

## Are there Jobs for Physicists to Do?

FOR several years there has been a running dispute between British universities and potential employers of science graduates in Britain about the suitability of university degrees for work in industry. Although in the past few months these complaints have been overshadowed by arguments about the Rothschild proposals for publicly supported research and by the general despondency of academics about the future of universities as such, they came to the surface again last week at the Physics Exhibition, when Dr D. T. Swift-Hook (see page 135) rehearsed some now familiar complaints about the difficulty of making use of physicists in commercial enterprises. The essence of the complaint is that university courses, undergraduate and postgraduate, are too academic in content and in character, that industry is unable to make ready use of those who leave universities and that it cannot in any case keep university graduates usefully employed at research and development for more than a decade or so. In short, as Dr Swift-Hook puts it, the output from the universities must be more "goal oriented" and more flexible. In his address at the Physics Exhibition, he also lent support to the notion that scientists differ from other people in being too old at thirty. By doing so, he seems to have echoed the unfortunate recommendations of the CBI committee some months ago which stated that the durability of university-trained scientists in industrial life is so short that those who enter government service must be employed on short-term contracts.

Complaints like these against the present system of university education are inappropriate if only because they overlook the intricacy and the subtlety of the problems which must be solved. First of all, it is as well that those who complain of the inutility of university scientists should remember that there were very few complaints when, not so long ago, the whole of what is now called the nuclear power industry was created in a few short years by people from the universities. In the same spirit, it should be remembered that only a few years ago, industry was thanking its lucky stars that the universities were able to supply large numbers of trained scientists in strictly academic disciplines who were nevertheless able to convert themselves quickly into men and women capable of programming computers and even, on some occasions, designing them. It is also important that the universities themselves and the sponsors of university research such as the Science Research Council have in the past few years taken important steps to liberalize university courses in such a way as to make them more suitable for the ultimate needs of industry. The Science Research Council has encouraged industrially oriented courses, especially at the postgraduate level. The universities themselves have broken new ground with courses which combine scientific study and such things as economics. They have also been several important innovations in the creation of links between particular universities and particular industries and groups of industries.

Those who complain that the graduates of the universities are unsuitable for industry should at least acknowledge that this is constructive work.

A more substantial complaint than that which Dr Swift-Hook rehearsed last week is that industry does not in any case know what it wants. Some industrial laboratories are still foolishly supposing that new entrants from the universities must be helped to make a painless transition by being thrown into what is for practical purposes an industrial simulacrum of a university laboratory—the trick then is to hope that the sheer rough and tumble of industrial life will set mercantile juices running in the veins of academics. Others, for all their protestations, still regard industrial development as if it were a kind of service to industry and not an essential part of it. The result is that new entrants find themselves required to exercise their skill on tasks which have no obvious relevance either to the profitability of the enterprise concerned or to the career of the industrial scientist. Who then can legitimately complain if new graduates entering industry discover that what they have been taught about economics or management is no more relevant to their new occupation than, say, the theory of numbers? This is why it is entirely unreasonable that industrial companies should expect to be able to make fuller use of their scientifically qualified recruits until they have devised some way of integrating research and development more closely with the commercial objectives to which, on paper at least, their whole enterprise is geared.

Career prospects follow on from this. If it were true—and it is not—that scientists are played out at thirty, does it not follow that industry would be better advised to put its research and development out to contract and to recruit only those who can make a career in management from the start? If Dr Swift-Hook believes what he says, then surely it is an abdication of an organization's responsibility to its employees that it should recruit large numbers of young men and women without being able to offer them a continuing career. If, as is the case, scientists and engineers differ very little from other highly trained people in their capacity to adapt to changing circumstances, does it not follow that industry should be more ready to blend research and development with commercial management? To be sure, one consequence would no doubt be that large industries would have to become more overtly cellular in their internal organization but that, surely, is necessary on commercial grounds as well as in the interests of their employees.

## How Big is China?

ONE of the uncovenanted benefits of the welcome improvement in relations between mainland China and the outside world, marked in the past few weeks by the visit to China of President Nixon and the reestablishment of



diplomatic relations between Britain and China at the level of ambassadors, is that there may soon be more reliable information about the population of China, one of the demographic conundrums of the past few decades. It is therefore helpful if a little adventurous that the Population Reference Bureau in the United States should now have published a survey of what is known about the population of China and has chanced its arm with some cautious estimates of what the truth may be. The problem of the Chinese population is of course crucial for those who wish to know how thickly populated the world may be in the decades ahead. After all, the Chinese population is estimated in the United Nations Demographic Yearbook for 1970 to lie between 732 and 828 million—well over twenty per cent of the population of the world as a whole.

The difficulty of estimating the population of China stems not merely from the fragmentary character of the information about the Chinese population which has been published but from uncertainty about the accuracy of the censuses which have been carried out. The census of 1953, the last complete enumeration of the population of China, was carried out by unconventional methods and the total population then enumerated—583 million—may have been substantially in error. Clearly there are immense difficulties in counting such a large population and in 1953, the work of the census was spread over several months. And nobody can tell how much it was affected by the unwillingness of Chinese families to register women and children in previous censuses, the former on grounds that Women's Liberation would disapprove of and the latter on the grounds that registration is unlucky.

Between 1953 and 1957, the total population figures were published annually in China, but it appears that these were based not on registrations of births and deaths but on assumptions about the rate of growth of the population, assumed to have been 2.1 per cent per year in 1952. There has never been an official description of the age structure of the Chinese population, nor official estimates of birth and death rates for the population as a whole. But according to the Population Reference Bureau, until the early 1950s at least, the Chinese population was characterized by the high rates of births and deaths now found in all developing countries. In the first half of this century, the rate of increase of the population of China appears to have been 0.5 per cent a year, which implies that the acceleration of growth rate reported by the Chinese authorities in the early 1950s is primarily a consequence of a declining death rate.

What has happened since? The chances are that the decline of death rate has continued and may now be comparable to that in industrialized countries of the west or possibly a little higher, perhaps 1.5 per cent a year. The Population Reference Bureau estimates that the birth rate would have remained constant at least until the early 1950s, but that the campaign to reduce the fertility of the Chinese population which began in the late 1940s will have had a powerful effect on reducing the birth rate. The bureau estimates that before the Revolution, the birth rate would have been 4.3 per cent a year and that it might have been reduced by the mid-1950s to 3.8 per cent a year and to 3.2 per cent a year in the mid-1960s. In short, if these estimates, which are necessarily crude, are valid, China should at present be a long way along the road to the balance between birth and death at levels

of birth rate and death rate which are much more like those found in advanced societies than in the China of the 1940s.

What does this imply for the future population of the Chinese mainland? The spread of almost 100 million between the upper and lower predictions put out by the United Nations is a measure of the uncertainties which must attend any answer to this question. This is why a great many demographers and their political interpreters will await with great interest any estimates that may soon be made available from official sources in Peking. So great is the uncertainty that there will be great value even in demographic statistics which are based not on complete censuses but rather on population surveys in limited regions of mainland China. One curious consequence of the cultural revolution, at least so far as demographers are concerned, is that the organization of large parts of the Chinese population into a communal framework, with each unit responsible for keeping its own statistics of births and deaths, implies that there must be readily available statistics for isolated pockets of population.

It will be of great importance to see what influence if any the cultural revolution will have had on birth and death rates. And there will be some wonder outside China that a government which is supposedly draconian in its methods of government should have been able to order its affairs without knowing how many people it controls. Or is it that the lack of demographic data from China is yet another sign that the government of China has been compelled by circumstances and size to regulate its affairs by devolution?

## Neglect of the Future

THE British Post Office has lost the friendship of its dwindling band of supporters by making public its plans for a further increase in the cost of telephones in Britain. The cost of installing a telephone is to be increased. So is the annual rental of a telephone line. But there are no compensating reductions in the cost of making telephone calls. One of the absurd reasons given for the extra charge for connecting a person to the telephone system is that the demand for new telephones continues to grow as quickly as the Post Office can install new lines. The result, no doubt, will help to moderate the demand for telephone service in Britain and will bring it more nearly into line with the capacity of the Post Office to install new equipment. But it is hardly consonant with the declaration of the British government and of the Post Office Corporation that the reconstituted organization would function exactly in the way that a private company does.

The charges made for the telephone service have an important bearing on the exploitation of an important new technology and on the likelihood that the fullest use will be made of it in the years ahead. The first thing to say is that it may be entirely proper that the annual rental for a telephone should be a larger component of the price which users of the system pay. For one thing, the Post Office has in the past few years been spending more and more heavily on capital equipment. The depreciation of its assets accounts for close on a quarter of its total revenue, while interest to be paid on sums of



money borrowed in recent years from the Treasury is a comparable if smaller sum. By comparison, the operating cost of the telephone service is only a little more than half of the total cost. And there is good reason to expect that expenditure on capital equipment will be a still larger proportion of total expenditure in the years immediately ahead. In the installation of a new telephone line, it is clear that the capital costs may be now very large, which implies that it is only right that telephone users should pay a substantial rental for the privilege of being connected to the system. But once a person is connected to the telephone system, there are the strongest possible reasons why the Post Office should encourage them to make the fullest use of the system. Britain is notorious for the numbers of people who make very little use of the telephones which clutter up their living rooms. The way to do this, as the Post Office Corporation should know by now, is to match the prices charged for using the telephone system with the marginal costs which it incurs itself. In present circumstances, when there has been considerable progress (but not before time) towards an integrated automatic telephone service in Britain, there is probably a case for a substantial reduction of the prices charged for telephone calls. Is it possible that the Post Office Corporation has neglected to make them now simply because it is expecting that inflation will soon make up the difference?

The notion that new telephone subscribers should pay a substantial price for being connected to the system is more debatable. Here, it is true, the Post Office may be arranging for users to bear the true cost of the service with which they are provided. This, however, is a practice which commercial companies would probably avoid like the plague, for there are good business reasons for arranging that as many people as possible should become potential customers. Not merely will they become contributors to the cost of the system as a whole, but people already connected to it will find the system more valuable—there will be more people to telephone. There are also important social reasons why the cost of being connected with the telephone system should be as low as possible. In Britain as in other industrialized societies, old people, sick people and isolated people need access to a telephone if they are to be fully integrated with the rest of society. In the United States, this need has been recognized by the decision in the past few years that some parts of the monthly telephone bill should be chargeable to welfare payments to the disadvantaged. Some weeks ago, Dr Michael Young and his associates were asking for a similar arrangement in Britain. Why has the Post Office Corporation moved, to all appearances, in the other direction?

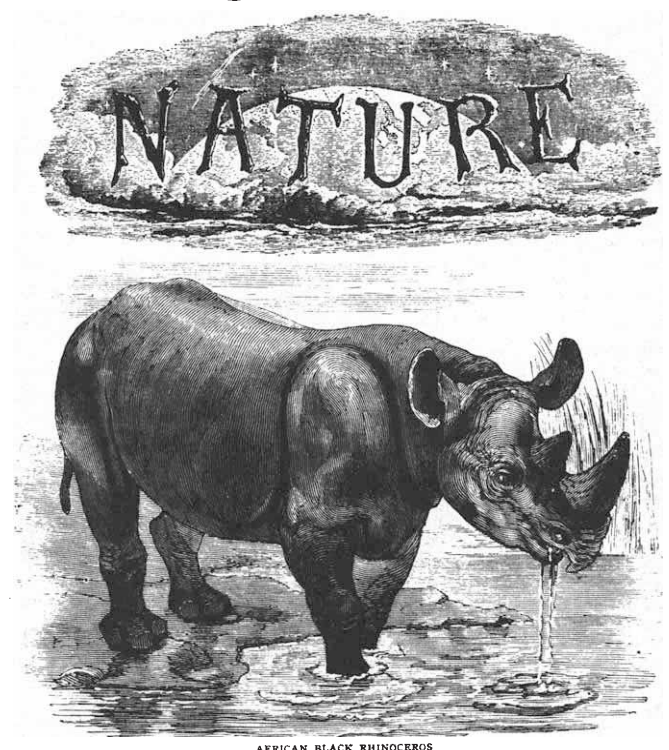
Complaints like these against the present policies which tend to discourage the use of the telephone, by all tests a beneficent technical development, can be matched against the prospect for the future, for which the Post Office Corporation is not entirely to blame. To be sure, because of indecision and bad decisions about the installation of electronic telephone exchanges in the 1960s, it will be the best part of a decade before the Post Office is properly equipped to make the fullest use of new technology. By then, other needs for telecommunications than mere telephone service will be crying out for capital investment. The most important development, already plainly foreseen, is that coaxial cables will be widely used for distributing television signals between offices and to

people's homes. But once there is a system of coaxial cables connecting individual subscribers comparable in complexity with the present telephone system, many other uses will come to the fore. Facsimile transmission is one obvious possibility, which may in due course provide the Post Office itself with a means of making the postal service profitable. In the past few years, even in Britain, there have been cautious developments in this direction. The new town of Cumbernauld, for example, is wired with coaxial cables, while in the past few months the British government has sanctioned a cable television service in Greenwich.

It is, however, most unreasonable to try to meet the future in such a piecemeal fashion. One difficulty is that the government is apparently unsure about the future of coaxial cables for telecommunications. Certainly it has no policy towards their use. But is it not essential, now that it is clear that it can only be a matter of time before coaxial cables play an important part in telecommunications, that the Post Office Corporation should set its mind to the design of an integrated system of coaxial cables?

It goes without saying, of course, that such a development would greatly affect the government's policy on broadcasting as a whole, but no harm would in the long run be done if there grew up what is for practical purposes a system of broadcasting by telecommunications. After all, it can only be a short time—with the BBC charter due to be revised in 1976—before the government finds itself urging on public and commercial television broadcasters alike that the time has come to separate the making of television programmes from the broadcasting of them. It is towards such opportunities that the Post Office Corporation should be directing its attention.

## 100 Years Ago



AFRICAN BLACK RHINOCEROS

From *Nature*, 5, 427, March 28, 1872.

## OLD WORLD

# Departments Respond to Rothschild

LAST week it was the turn of government departments to give an account to the Select Committee on Science and Technology of the way in which they organize their own research. The select committee was clearly anxious to establish to what extent the departments are implementing the customer-contractor principle, and how accountable they are for the research they do—especially in view of Lord Rothschild's proposal to reallocate about £28 million of research council funds to the appropriate departments.

Inevitably it was this reallocation, and the basis on which it was advocated by Lord Rothschild in his contentious Table 4, which first came under scrutiny. But Sir William Pile, permanent secretary at the Department of Education and Science would only say that the £28 million represented Lord Rothschild's personal view of the value of the applied research which the research councils carry out.

Sir William said that the amount of research done by the DES as opposed to that carried out by the research councils—was quite small (see Table 1) and chiefly educational in character, so it would not be necessary for the department to appoint a controller of research and development to act as the "contractor". Two of the other departments expressed the view that a single controller would not be appropriate. Sir Philip Rogers, permanent secretary at the Department of Health and Social Security, envisaged two controllers for his department, because he thought that it would not necessarily be advisable for the chief executive of the Medical Research Council (whom Lord Rothschild would have as sole controller for the DHSS) to be the contractor for research directed, for example, to the improvement of the social services. And Sir Basil Engholm, permanent secretary at the Ministry of Agriculture, Fisheries and Food, said he thought that more than one controller would be essential in MAFF in view of its quite distinct interests in research on agriculture and fisheries, for example. In its memorandum to the committee, the Department of the Environment says that it has set up a body called the Research Contractors' Board which comprises the directors of the Department of Environmental research establishments and the Director of Research Requirements, who in turn is part of its embryo chief scientist organization.

**Table 1** Expenditure on Research and Development by some Government Departments

|                                             | £ million |
|---------------------------------------------|-----------|
| Department of Education and Science         | 0.47*     |
| Department of Health and Social Security    | 9         |
| Department of Trade and Industry            | 201       |
| Department of the Environment               | 16        |
| Ministry of Agriculture, Fisheries and Food | 6         |

\*Excluding research council expenditure.

Sir Robert Marshall, Secretary (Industry) at the Department of Trade and Industry, said that the DTI was in the process of setting up seven requirements boards, each led by the appropriate DTI policy division, which would form part of the chief scientist organization and would commission work on the department's account in its own research establishments, the Atomic Energy Authority, universities and industry. The Department of Health and Social Security says that it intends to "appoint a chief scientist, on a temporary basis of from three to five years, together with a small nucleus of other scientists, part-time and also temporary . . .", and the Ministry of Agriculture, Fisheries and Food has in mind "a chief scientist and two deputies who would have the responsibility, in conjunction with the policy and economic divisions concerned, of framing and keeping under

review the agriculture and food research programmes . . . whether carried out by the ministry's own research establishments or by the Agricultural Research Council".

Most of the witnesses said that the research done in their departments is accountable to the relevant minister, and Sir David Serpell, permanent secretary at the Department of the Environment pointed out that there is also accountability to parliament through the departmental votes. Sir Basil Engholm said that it was not his ministry's practice to lay a report on research and development before parliament because it was too intimately integrated with the work of the ministry as a whole.

Members of the select committee—in particular Mr David Ginsberg—felt that the research councils are, in practice, more accountable for their research work than government departments.

## EDUCATION

### Women's Liberation in Education?

STATISTICS released yesterday by the Department of Education and Science show that there were 13,000 more students enrolled for full-time further education courses in England and Wales in 1970 than there were the previous year. This increase, however, was partly offset by a decrease of 7,400 in the number of students following part-time courses. (*Statistics of Education; Vol 3, Further Education*, HMSO, £1.90.)

These statistics apply to non-university establishments—the polytechnics, colleges of education, technical colleges, colleges of art, agriculture colleges and other Local Education maintained establishments. The total numbers in further education establishments in November, 1970, were 3,181,200, which was a 3.2 per cent increase over 1969. This increase reverses the slight decrease experienced in the previous two years. The change comes mostly from a nine

per cent increase in the number of students registered for sandwich courses. But there was also a five per cent increase in students attending evening institutes. Of particular interest is the fact that ninety per cent of the increase in enrolled students was due to women. The report says that the increase in women students and a decrease in men reflects a trend that has been going on during recent years: in late 1970 there were 1,528,200 women and 1,653,000 men registered for courses. Between 1961 and 1970 the numbers of women students increased by 44.9 per cent while the enrolment of men increased by only 20.7 per cent in the same period.

Another trend that is evident is the increase in the average age of students in further education. In 1960 44 per cent of students were over 21, whereas in 1970 the number had increased to 50 per cent.



## CAREERS

## Where Will They Go?

THE training of high energy physicists and molecular spectroscopists in universities "makes them worse than useless" in industry, according to Dr D. T. Swift-Hook, head of electrical research at the CEBG laboratories at Marsham. Dr Swift-Hook, who was addressing a meeting at the Physics Exhibition last week on careers for physicists, favoured a PhD which was "goal oriented" and he would like to encourage more PhDs to be done within industry.

A physicist entering industry with a PhD takes several years to catch up with salary that he has lost because he did not sell his soul to some company after his first degree. Dr Swift-Hook did admit, however, that PhDs usually command a salary some 10-15 per cent higher than a physicist without such qualifications, but an MSc graduate, in general, does not do better than a BSc. Dr Swift-Hook attributed this to the underlying motives of students who study for MSc degrees—he suggested that students who embarked on such courses were mostly covering up a bad first degree or they were PhDs who had failed to make the grade.

The Central Electricity Generating Board restricts its recruiting to people under the age of 26. The reason for this, according to Dr Swift-Hook, is that the research worker is past his prime at thirty and that research managers have great difficulty keeping the average age of scientists in the laboratories below thirty without having additional complications introduced by recruiting people who are in their late twenties. Dr Swift-Hook said that it was a problem of what to do with the older scientist, as not all of them had the potential to become managers.

Of more benefit to physicists and career advisors was the talk given by Mr P. L. Flowerday, assistant secretary of the Institute of Physics, who attempted to pinpoint areas where there would be vacancies for this summer's crop of graduating physicists. There are 26,000 physicists in Britain at present, a number that is being added to at the now constant rate of 2,200 fresh graduates a year. Of these nearly 10,000 are in education in one form or other and this year Mr Flowerday estimates there will be a need for 500 to 750 more physicists in this sector. The public sector which includes the Atomic Energy Authority, Research Associations and the Health Service and has 5,000 physicists in its ranks is experiencing a period of stagnation and there is little prospect of many physicists finding jobs here this autumn.

There are 8,000 physicists in industry at present and Mr Flowerday reckons that only 500-600 more new physicists

will be needed this year and these will probably have to diversify into fields not normally filled by physicists. The remaining graduates, said Mr Flowerday, will have to find employment in fields such as commerce and it is here that at least 500 graduates will have to deploy their talents this year. The situation, according to Mr Flowerday, is no worse than last year and returns of employment of 1971 graduates showed, last December, that no more physicists were unemployed then than there were in 1969 or 1970. Prospective graduate physicists should heed Mr Flowerday's closing words when they look for employment: "be prepared to diversify and good luck".

## CHEMICAL EDUCATION

## ICI Makes a New Move

LAST week saw the publication of the first eight of a series of booklets written jointly by chemistry teachers and chemists from ICI with sixth form project work in mind. ICI says that the booklets are designed to form the basis of a kind of personal library, which might grow as samples, photographs and articles are collected by sixth formers according to their individual interests.

One of the guiding spirits behind the scheme has been Dr Duncan Davies, general manager in charge of ICI's research and development effort. Dr

Davies said last week that it is not paradoxical to find ICI involving itself in chemical education in schools at a time when jobs for chemistry graduates are in short supply, because "chemistry is a socially central subject" and good chemistry teaching therefore needs to be encouraged. He also emphasized that ICI's position in the new scheme is as a participant and not as a patron, which is why a slightly subsidized charge of £1 is being made for the first group of eight publications. This also means that reprinting or revising as necessary is not too strongly dependent on the funds available for educational projects at any future time.

The eight booklets—on subjects ranging from polymers to spectroscopy—were the fruits of three days of concentrated work at the University of York when the ICI schools liaison officer, Mr R. Finch, once a chemistry teacher himself, gathered together some thirty scientists, teachers and lecturers. The result is what Mr Finch hopes is "a collection of up-to-date material written at the right level and directly useful in the teaching of sixth form chemistry". He describes them as "project starters rather than encyclopaedias". The first reaction of Mr Martyn Berry, head of the chemistry department at Sidcup and Chislehurst Grammar School for Boys, was that the new publications would probably be a useful complement to Nuffield A-level chemistry courses.

## COMMON MARKET

## Facts and Figures for the Future

WITH less than ten months to go before Britain, parliament and the French electorate willing, joins the European Economic Community, the data given

in the following table shows how the expanded community will fare in the world economic league table. The figures are for 1970.

Table 1. A Comparison of Economic and Industrial Power\*

|                                                                          | Six         | Ten     | USA     | USSR    | Japan    |
|--------------------------------------------------------------------------|-------------|---------|---------|---------|----------|
| Population (in millions)                                                 | 189.8       | 257.2   | 205.4   | 244.0   | 103.5    |
| Gross National Product (in \$10 <sup>9</sup> )                           | 485.2       | 637.4   | 933.3   | 288†    | —        |
| Imports (percentage of world total)¶                                     | 30.3 (18.3) | 41.0    | 13.7    | 4.0     | 6.5      |
| Exports (percentage of world total)¶                                     | 31.8 (19.2) | 41.2    | 15.5    | 4.6     | 6.9      |
| Production of primary energy (million tons of coal equivalent)           | 330.8       | 520.4   | 2,151.4 | 1,386.1 | 71.4     |
| Internal consumption of primary energy (million tons of coal equivalent) | 845.8       | 1,235.8 | 2,250.6 | —       | 379.6    |
| Mineral oil production (million tons)                                    | 391.7       | 504.2   | 565.5   | —       | 159.7    |
| Electricity production (10 <sup>6</sup> MWh)                             | 580.4       | 909.2   | 1,738.1 | 740.9   | 350.6    |
| Steel production (million tons)                                          | 109.2       | 138.9   | 122.1   | 116.0   | 93.3     |
| Automobile production (10 <sup>6</sup> units)                            | 8,029       | 9,670   | 6,550   | 348     | 3,179    |
| Railways (millions of passenger km)                                      | 120,171     | 155,748 | 10,568  | 266,300 | 181,921‡ |
| Energy consumption per capita in industry (kWh)                          | 1,672       | 1,736   | (3,300) | (1,896) | (1,860)  |
| Energy consumption per capita for other purposes (kWh)                   | 1,070       | 1,387   | (4,000) | (698)   | (1,228)  |
| Passenger vehicles per 10 <sup>3</sup> inhabitants (January 1, 1971)     | 220         | 218     | 432     | 7       | 85       |
| Television sets per 10 <sup>3</sup> inhabitants                          | 216         | 231     | 399     | 127     | 214      |
| Telephones per 10 <sup>3</sup> inhabitants                               | 185         | 203     | 567     | 50      | 194      |

\* Data from Commission of the European Communities. † Net material product. ‡ 1969.

¶ Figures for the six and the ten include internal imports and exports. The present external figures for the six are shown in brackets.

## GEOPHYSICS

## Three Leagues Under

from our Soviet Correspondent

THE latest geophysical project envisaged in the Soviet Union is the drilling of a borehole into the Earth's crust—to reach, it is hoped, a depth of 15 km—that will be more than twice as deep as any previous borehole.

This project, to be undertaken by a team of scientists from Moscow, Baku and Sverdlovsk, is led by Academician Ali A. Alizade, Director of the Baku Scientific Research Institute of the Oil Industry. The site of the proposed bore is in the Saatlov region of southern Azerbaijan. The equipment to be used is basically the standard heavy-duty 'Uralmash' drilling rig, equipped with drilling motors, drill-tubes and the latest type of bits.

In accordance with current Soviet policy, by which all research is, in theory, directed towards the needs of industry and the national economy, Academician Alizade, when interviewed by *Izvestiya*, stressed the practical benefits of such a project. In the course of drilling, he explains, it is likely that "petroleum and gas, deep-level heat, thermal waters, and solid ores, including polymetallic complexes" may be encountered. Yet this is, clearly, to be more than an ultra-deep mining project. The research team, says Alizade, are particularly interested in the physical state of the "riches" encountered "which lie constantly in a zone of high temperatures and pressures". More general aims of the project include "the study of the deep-level structure of the Earth, investigation of the problems of the petroleum- and gas-bearing capacity of mesozoic deposits, and the plotting of a detailed cross-section of the Earth's crust".

A pilot bore to the depth of 5 km is already in progress, and has so far reached a depth of 1.6 km. With the main bore, a barrier of crystalline-granite and basaltic layers is expected deeper than 7.5 km and, apart from this, says Academician Alizade, the team are prepared for "all possible manner of surprises, which until now have remained hidden in mystery".

## NATURE RESERVES

## Places to Visit

THE Nature Conservancy announced last week that it has acquired a further 2,761 acres thus bringing the total area of Britain's nature reserves up to 271,404 acres. Some of the additional land will form one new reserve at Walberswick in Suffolk, and the remainder will go to enlarge the reserves at Braunton Burrows, Devon, Old Winchester Hill, Hampshire, Cavenham Heath, Suffolk,

Dyfi, Cardiganshire and Nant Irfon, Breconshire.

The new reserve at Walberswick will cover 1,211 acres, chiefly heathland, marshes and woods. Most of the reserve was signed over to the Nature Conservancy by Sir Charles Blois and the conservancy had only to purchase 95 acres. The attraction of this new reserve is that many wildfowl and wader are common visitors and shellduck are to be found there all the year round.

The largest addition announced is a further 932 acres to Braunton Burrows National Reserve in Devon; this brings the total within the reserve to 1,492 acres. Most of the new area comprises sand dunes which are rich in plant and animal life. Another eleven acres have also been added to the chalk downlands of Old Winchester Hill National Nature Reserve which is famous for its juniper scrub and wild flowers. The addition of 39 acres to the reserve at Cavenham Heath brings the total acreage to 376.

This addition is an area called Ash Plantation—an example of damp mature fen woodland which provides a striking contrast to the dry heathland that lies nearby. Ash Plantation is rich in ferns and mosses, and it also provides a refuge for a herd of Roe deer.

Two reserves in Wales are also being enlarged with 445 acres have been added to the reserve on the estuary of the Dyfi river. The most important addition consists of 208 acres of peatland at Cors Fochno which will give botanists the opportunity of studying one of the few remaining examples of a "raised" bog. Cors Fochno is also a haven for white-fronted geese and other wildfowl. The addition of 120 acres to the Breconshire reserve means that the reserve now covers the entire western bank and valley slope of the river Irfon near the village of Abergwesyn. The attraction of this reserve is that it is an area of sessile oak woodland, and many mountain streams and rocky crags are now included within it.

## RESEARCH ASSOCIATIONS

## Change in Role Prescribed by CSII

RESEARCH associations should concentrate their efforts on work directly commissioned by their members rather than on general cooperative research. This Rothschildian recommendation emerges from the most constructive piece of work yet undertaken by the Centre for the Study of Industrial Innovation which has turned its attention from the heady objectives of trying to identify what makes innovations succeed and fail (see *Nature*, **235**, 366; 1971) to the bread and butter job of examining the work of research associations.

The forty research associations, whose activities range from Hosiery to Drop Forging, spent 2.2 per cent of the research budget of industry in 1968/69. This amounted to £16 million of which just over £4 million came from government grants. But since 1968 the restriction of grants from the Department of Trade and Industry has put the research associations under pressure and there is now a £4 million ceiling to government support of the associations.

In spite of this the expenditure of the RAs still grew fractionally faster than that of private industry during the 1960s.

But the RAs have come in for much heavy criticism from industry in recent years, and it is clear that some of the criticism has been justified.

Research associations were founded to perform cooperative industrial research but the fact that many companies feel that the associations do not meet their immediate needs is reflected in the figures for directly commissioned research undertaken by the RAs. In 1963 it formed 14 per cent of the RAs

income and in 1970 only 25 per cent. This figure is rising but the report points out that it must do so if RAs are to remain a viable source of technical help for industry.

The RAs which tend to be most effective, according to the report, are those in areas with less rapidly advancing technologies—for example in the clothing, construction and non-ferrous metals industries, where the work done by the research associations accounts for 10 per cent of all the research and development done in these industries.

The report also calls for a review of the government's grant system. Grants should be given for more specific purposes, according to the CSII, perhaps to finance a core of basic work in the research associations, as distinct from the customer-contractor work that should form the bulk of their research.

But while the past activities of the research associations do not emerge in a halcyon light from the CSII's scrutiny (although the institute is careful to point out that not all the associations are at fault), the industry which the associations are meant to serve does not emerge as perfect. Some of the dissatisfaction of industry with the associations is due to companies misunderstanding what the associations are for. The belief that paying the subscription entitles companies to a supply of attractive and instantly usable technical results is a mistaken one. The report says that each company should ensure that it in fact takes the necessary steps to derive the maximum value it can from membership of an association.



## NEW WORLD

## Technology Message Springs Few Surprises

by our Washington Correspondent

PRESIDENT NIXON'S first and long awaited message on technology was delivered to Congress last week. It turned out to be something of an anticlimax. It contained little that had not already been unveiled in the budget or in the State of the Union message, there were no new funding proposals and it was significantly devoid of schemes to stimulate industrial research and development by tax incentives or by relaxing anti-trust laws.

The message was, nevertheless, described by Dr Edward E. David, jun., the President's Science Adviser, as marking a watershed in federal science policy planning. Dr David argued, at a press briefing on the message, that it underlines the Administration's attempts over the past year to find new ways to stimulate civilian research and development and to use science and technology to help solve pressing national problems. It is the beginning, he said, of a science policy that is for the first time integrated with national policies.

Stripped of the political rhetoric which inevitably accompanies Presidential messages to Congress, however, the technology message principally outlines the chief civil science projects that soaked up most of the increases in the Administration's science budget. It also announces a few relatively minor new devices for stimulating industrial research and clearly underlines the importance that the Administration is attaching to the new technology incentives programme that will be conducted by the National Science Foundation and the National Bureau of Standards (see *Nature*, 236, 7; 1972). That programme, described by Dr David as the key to the effort to stimulate research and development in industry, pops up no fewer than six times in the message and its progress will clearly need watching for it could be the pilot for future investments by the federal government in industrial research.

The technology message is the fruit of the technology initiatives programme that was started in the autumn of 1970 by the Office of Science and Technology, and which came to prominence last summer when Mr William M. Magruder was appointed to the White House as Special Consultant on high technology projects. Mr Magruder's job was to provide the chief control over the technology initiatives project which, having started as a search for worthwhile technological projects likely

to lead to benefits for society, had branched into a more detailed look at various incentives for encouraging industries to invest in science and technology. At one time, the study was expected to produce a list of projects calling for expenditures of up to \$2,000 million in federal funds, but after they had been sifted by the Office of Management and Budget, and the Office of Science and Technology, the list had shrunk to much more modest proportions, accounting partly for an increase of some \$400 million in the federal budget for civilian science.



Edward E. David—treading carefully.

When Mr Magruder was appointed, the Administration was also toying with the idea of using the tax system to help stimulate industrial research and development. Indeed, in his economic message to Congress on August 15 last year, President Nixon announced that he had directed the Secretary of the Treasury to recommend to Congress in January new tax proposals for stimulating research and development. But last week's technology message contained no proposals for tax incentives and it seems from recent pronouncements by Mr George P. Shultz, director of the Office of Management and Budget, that the idea has been dropped, at least for the time being. Last month, Mr Shultz told reporters that the problem is to define what kinds of research investments should qualify for tax relief. If the definition is too broad, companies may receive large rebates for research they would have made in any case.

Another suggestion that was widely canvassed last summer and which seems to have fallen by the wayside is a relaxation of the anti-trust laws. Mr

Maurice Stans, then Secretary of Commerce, suggested during hearings on science, technology and the economy held in August by Mr John Davis's Subcommittee on Science, Research and Development, that anti-trust laws may be preventing companies from pooling research into anti-pollution devices. But the technology message does little more than restate present anti-trust policies, suggesting that any joint programme for research and development must be approached in a way that does not detract from normal competitive incentives of our free enterprise economy. Those who were suggesting that the recent departure from the Department of Justice of Mr Richard Maclaren, former chief of the Anti-trust Division, would pave the way for more liberal anti-trust policies, were therefore clearly mistaken.

The clear indication in the technology message therefore seems to be that the Administration has decided not to jump straight into the business of providing incentives for industrial research until it has a better understanding of how the innovation process works. And that is where the NSF's technology incentives programme comes in. Dr David suggests, for example, that we will not be rushing headlong into the full programme until the pilot incentives programmes being tested by the National Science Foundation have produced some tangible results. And that may take a year or two.

In the meantime, however, President Nixon's message contained a few relatively minor proposals that will be instituted before the foundation's programme bears fruit. He announced that he is taking action to permit the National Science Foundation to support applied research in industry when the use of industrial capabilities would be advantageous in accomplishing the foundation's objectives. On the face of things, however, this provision will not alter the foundation's activities, since under its new charter which was adopted in 1968, it is already allowed to support applied research in industry when so directed by the President.

Patent policies also came in for mention in the message, but again, not much change is envisaged from the new arrangements outlined in September last year (see *Nature*, 233, 6; 1971). The new arrangements allow private companies to obtain exclusive licences to exploit technologies developed under government contracts and the chief thrust now seems to be to make the new policy more widely known. Presi-



dent Nixon also announced that he will be instituting a new programme of research and development prizes, but Dr David admitted that no details have yet been worked out.

A more explicit announcement in the message is that the Department of Commerce will now be the focal point in the executive branch of the federal government for policies concerning research and development. The idea is that the staff of the commerce department will keep an eye on the technological strengths and weaknesses of US industries, and identify barriers to applications of new technology. An important input into the department's deliberations will be the NSF's technological incentives project, and a potentially important factor in the department's handling of its new role is that Peter G. Peterson, the newly appointed Secretary of Commerce, was one of the key figures in the development of the technology incentives project that led to last week's message.

A final point is the announcement of several steps to promote international cooperation in research and development. A review of US participation in the scientific and technological programmes of international organizations has been set in train, to make United States participation in these activities more effective, with even stronger ties to our domestic programmes. And there was also even a formal invitation for scientists outside the United States to share some of the new wealth devoted to cancer research: "I am inviting other countries to join in efforts in the United States, including the effort to conquer cancer at the unique research facilities of our National Institutes of Health and at Fort Detrick, Maryland," President Nixon said.

Where does this leave Mr Magruder and the technology incentives programme? Mr Magruder has been conspicuous by his absence at press briefings on the federal government's science budget and the technology message, both of which were conducted exclusively by Dr David, and just as there was widespread speculation that Mr Magruder's appointment signalled a decline in Dr David's influence, there is now speculation that Mr Magruder himself may be on the way out. And those speculations have gained momentum from the fact that at one time the technology message was expected to include a recommendation for the setting up of a special office in the White House to oversee future developments in the technology incentives programme. Mr Magruder would, of course, have been an obvious candidate to head such an office.

It now seems, however, that Mr Magruder will be staying on in the White House as a special consultant in technology, and that his chief interest

will remain with the technology incentives project. Because the Administration is treading carefully by insisting on gaining more knowledge of the innovation process before taking decisive action, there will clearly be no shortage of evaluation to do.

## CANCER

### Watson's Predictions

In his annual report as director of the Cold Spring Harbor Laboratory, James D. Watson has analysed the recently enacted National Cancer Act. His conclusion, an excerpt from which follows, is that much of the money earmarked for research will find its way into the provision of new treatment facilities. His appointment last week to the National Cancer Advisory Board will at least enable him to have a say in whether this prediction is carried through.

"The recent Congressional verdict to keep cancer research decentralized within the NIH framework strikes me as a wise decision. The administrative ball game thus is back where it started, with the NCI still the dominant agency controlling how cancer money is dispensed. And as before, the existence of scores of different advisory bodies will ensure that many fingers are in the pie. Admittedly it is so much a fatter one that its future distribution by the NCI administrators is very much in doubt. They could opt primarily for long-term results by allocating a significant portion of the money to learning the precise chemical details of human cells. Or they could blanket much more cancer therapy under the umbrella of 'clinical research' and construct a series of brand new hospitals, with emphasis on regions now lacking the hospital resources of major medical centres like Boston, New York, and Houston.

"Most likely the NCI (as would have the superagency if it had been created) will put their money largely on the short-term option. In doing so, they will optimistically argue that pre-existing methods of therapy might have to be only slightly modified to produce real dividends and that the best way to ensure that the right new protocols for treatment are devised is to create new hospital centres specifically designed to decide between alternative methods of therapy. But from cruel experience the leaders of NCI must inwardly fear that, barring the emergence of radically new ways to cure the major cancers, the chief effect of a massive new round of hospital building will be to provide more cheerful places to die. The main dilemma the clinician lives with is not his failure to keep abreast of new discoveries which could cure his patients but the essential absence, in even the

newest cancer hospitals, of effective ways to check virulent cancers like those of the breast and the lung. Setting up of speedy all-inclusive information exchanges to reshuffle current knowledge is likely to enrich only the stockholders of computer companies. But the average citizen, as well as his congressmen, will badly want some short-term result to show for his money and so will be reassured by the sight of a fine new edifice equating glimmer with more effective therapy. We must expect that much, if not most, of the new cancer money will buy patient care, not true research.

"Fortunately, the massive funds just voted by Congress mean that even a small share of the pie is a lot of money and most likely sufficient to create a large number of first-rate new research groups whose existence would be impossible without the new money. The real question thus becomes *will this happen or will the NCI leadership just pour out the money without any real plan of action?*"

### A Home for the VLA

THE National Science Foundation has found a suitable home for the Very Large Array (VLA) system of antennae for radio astronomy. The proposed site is in the New Mexico desert south-west of Albuquerque, in a large valley called the Plains of San Augustin. The New Mexico site was chosen from a list of thirty-four possibilities screened by a site selection team from the National Radio Astronomy Observatory, which will manage the VLA for the National Science Foundation, and the choice was confirmed by a committee of the National Academy of Sciences.

The site has to meet several criteria, including proximity to the Equator to give good coverage of the sky, a high altitude to decrease atmospheric interference, remoteness to minimize interference from radio noise and ground vibration, and a gradient along the thirteen mile long legs of less than two per cent. The proposed site also has the advantage that it is near a small town, Socorro, where there are schooling, shopping and other facilities. The proposal to build the VLA was made in the National Science Foundation's Budget request (see *Nature*, **235**, 244; 1972) and it will entail a total expenditure of some \$65 million. If Congress agrees to the proposal, the instrument may be partially operating in 1976, and fully operational by 1982.

## NEWS AND VIEWS

## Cloud Seeding Developments

WHEN Schaefer, in 1946, dropped powdered dry ice into a layer of supercooled cloud and an observer on the ground saw snow fall from the base of the cloud, he initiated the cult of cloud seeding which was to occupy and frustrate experimental meteorologists in many countries for the next two decades. The primary purpose of the experiments with seeding clouds was to increase the amount of rain from the clouds, or to cause them to rain if they would not otherwise have done so; more recently seeding has also had the aim of attempting to reduce the growth of large hailstones which can cause so much damage to crops in regions of the world prone to severe thunderstorms.

Apart from the obvious visible evidence of experiments like that of Schaefer, the early workers based their hopes on the assumptions that the presence of ice crystals in a supercooled cloud is necessary to produce precipitation, or at least to enhance greatly its amount, and that there are many clouds which lack naturally formed ice crystals. In regions which are deficient in rainfall there are often non-precipitating clouds so that it was an obvious possibility that the water shortage could be remedied by causing these clouds to rain. The supercooled water drops were made to freeze by introducing into the clouds either powdered dry ice or, more usually, crystals of silver iodide.

It soon became apparent that although some clouds could be found in a state suitable for seeding, that is, they could be made to shower when otherwise unlikely to do so, the amount of rain produced was trivial. When more extensive trials were carried out, it was difficult to compare statistically the rainfall from seeded clouds with that estimated to have occurred without seeding, either on the basis of long-term rainfall records, or in comparison with control areas. It eventually became clear that any increase in rainfall over a significant area as a result of cloud seeding was so small compared with the natural variation that any experiment seeking to prove the increase statistically would have to be run for many years, perhaps a decade or two. At present, therefore, there is little evidence to support the idea of increasing precipitation by seeding, and indeed there is certain evidence to suggest that seeding can actually produce a decrease.

Now that experiments cannot give a decision on the value of cloud seeding, meteorologists have recently been turning their attention to the theory of precipitation production, and the theoretical effect of freezing the supercooled cloud droplets. These investigations are chiefly concerned with the two hypotheses previously stated on which the effect of seeding is based. Because cloud seeding is carried out by introducing into the cloud artificial ice nuclei, it is clearly of importance to identify and count those particulates in the atmosphere which act as natural ice nuclei.

It is in principle simple to count the number of ice nuclei in a volume of air by cooling the volume to pro-

duce a supercooled cloud and counting the ice crystals which are then produced. Unfortunately there are many experimental difficulties that have not been completely resolved. There seem to be fewer than  $10^2 \text{ m}^{-3}$  active ice nuclei at temperatures above  $-15^\circ \text{C}$ , rising to perhaps  $10^6 \text{ m}^{-3}$  or more when the temperature is below  $-30^\circ \text{C}$ . Unfortunately the numbers of ice crystals observed in clouds can be up to one thousand times the number of ice nuclei, and this can be explained either by the rapid splintering of crystals or by the failure to detect large numbers of very small nuclei, of radius perhaps  $10^{-2} \mu\text{m}$  or less.

Although it is now fairly easy to count the numbers of ice nuclei, except perhaps for the smallest, it is far more difficult to determine the material of which they consist, and their places of origin, because they are a minute fraction of the total number of atmospheric nuclei the concentration of which is  $10^9 \text{ m}^{-3}$  or more. It is possible to test nuclei derived from natural sources for their ice nucleating ability; the only substances until now found to be of any consequence are silicate minerals of clay, of which the most common is kaolinite. Because kaolinite only constitutes a very small proportion of the Earth's dust surface, it might well occur in atmospheric nuclei in the amount required. Electron diffraction of the particulates in atmospheric ice crystals shows that a very high percentage of such particles are clay. Clay, however, is easy to identify by this technique, and these particles could as easily have been collected by the crystal after its nucleation. Now Schnell and Vali, on page 163 of this issue of *Nature*, put forward the idea that decaying vegetation can provide ice nuclei which are active at comparatively high temperatures. Should their preliminary investigation be substantiated and if it can be found in what size ranges and concentrations such nuclei are put into the atmosphere, the problem of the atmospheric ice nuclei could be one step nearer solution.

It is sometimes observed that ice nuclei are more abundant in the high troposphere than near the surface. This has led E. G. Bowen (*Aust. J. Phys.*, **6**, 490; 1953) to put forward the idea that at least some ice nuclei are meteoritic dust particles. He contended that mean rainfall records from different regions in the world show peaks which can be related to meteor showers, with a time lag between the shower and the rainfall peak of about 30 days. Unfortunately there are many objections to such a theory. There has been considerable dispute about the statistical significance of the peaks in the rainfall data in view of the large random fluctuation in rainfall and the serial correlation existing in the data. Only meteoritic dust particles smaller than  $10 \mu\text{m}$  reach the troposphere. Although these  $10 \mu\text{m}$  particles should take about 30 days to reach the level of cloud tops, the far more numerous smaller ones will take much longer, and should therefore arrive at a fairly uniform rate. Even ignoring these two objections, it still has to be assumed that the amount of rainfall depends on the actual concen-



tration of ice nuclei, and that meteoritic dust constitutes a high proportion of these; both these assumptions are of doubtful validity.

As a further complication, it has also been claimed that the mean rainfall has an oscillation in phase with the Moon, and it has been suggested that there may be a lunar modulation of the incoming meteoric particles. Although such ideas cannot be rejected out of hand, any theory linking ice nuclei to rainfall still has to explain the physical processes involved in that linkage, against the presently accepted notion that the actual concentration of ice nuclei, within some broad range, is irrelevant in the precipitation process. Nevertheless it must be admitted that the origin and nature, and perhaps even the concentration, of ice nuclei are at present still in dispute.—From a Correspondent.

## Rotating Rhodopsin

Two articles in last week's issue of *Nature New Biology* (March 15) may in the future be recognized as landmarks in the evolution of ideas of biological membrane structure. Both concern the physical state of rhodopsin in the membrane of the retinal rod outer segment. Rhodopsin is the preponderant, indeed the only major, protein of the rod membranes, which are arranged like a stack of pancakes lying perpendicular to the long axis of the rod. It has been known for some years that the rod is strongly dichroic in respect of rhodopsin absorption, the polarization being essentially perpendicular to the axis. Because the electronic transition moment of the chromophore, the 11-*cis* retinal, conjugated to the protein, is in its long dimension, it follows that these chromophores lie roughly perpendicular to the rod, and that the protein, which is probably more or less spherical in shape, has a precisely defined orientation relative to the rod axis.

Seen end-on, however, the rods have no dichroism, which can only mean that the chromophores (and therefore the protein molecules) are randomly oriented with respect to rotation about the line of the rod axis. Moreover, in 1960 Hagins and Jennings reported a finding that has lain in the literature as a subject of uneasy conjecture, namely that no dichroism is induced after partial bleaching by exposure to a flash of plane polarized light. This was at first sight astonishing, for one would have supposed that after elimination of a proportion of the chromophore axes which lie most nearly along the direction of polarization of the bleaching light, the residual chromophores would tend to be oriented at right angles to this plane. Hagins and Jennings had two reasonable explanations, however; the one with instant appeal to spectroscopists, who have always had an inclination to dabble in this system, is that the polarization is dissipated by excitation transfer between closely lying rhodopsin molecules within the lifetime of the excited state. The second is that the molecules are free to rotate in the long axis direction of the rod, and their orientation becomes randomized within the time that elapses in making the measurement.

That the second explanation is the correct one has now been conclusively established by Brown (*Nature New Biology*, **236**, 35; 1972), by the simple expedient of cross-

linking the system with the bifunctional reagent, glutaraldehyde, so as to inhibit rotational motion. The chemistry of the pigment is unaffected by this reaction, and, the concentration of rhodopsin in the membranes being very high, intermolecular cross-links are readily formed, so that it becomes impossible to extract protein from the rods. After such treatment, partial bleaching of the retina with polarized light leaves the rods markedly dichroic to end-on view, the dichroic ratio increasing with the extent of bleaching. The monofunctional reagent, formaldehyde, engenders no such effect. A further experiment, to exclude any important contribution of energy transfer towards the depolarization of the absorption of normal retinas, involved the prior bleaching of the overwhelming part of the rhodopsin with unpolarized light. This treatment leaves the concentration of unbleached rhodopsin so low, in other words the separation between molecules so large, as to exclude significant energy transfer (a process which depends on the inverse sixth-power of the separation of the chromophores); yet the response of the rods to polarized light remained unchanged.

In the accompanying article (*ibid.*, 39), Cone has enlarged on these findings by actually measuring the rate of decay of the instantaneous flash-induced dichroism. In the short time scale of these experiments the bleaching of the rhodopsin proceeds only as far as one of the intermediate spectroscopic states of the pigment, in which the 11-*cis* retinene has been isomerized to the all-*trans* state, but has not yet been released from the protein. One may then in principle observe either the dichroism due to the appearance of this new species, or that of the residual unchanged rhodopsin, by appropriate selection of wavelengths, to the extent that the two spectra are separable. Dichroic ratios of up to two are in fact generated by polarized flashes. If, on the other hand, control measurements are made at an isosbestic point for the rhodopsin and the product, their dichroic contributions, if they decay at the same rate, should be equal and opposite, so that no net dichroism results. This condition is fulfilled for the first two bleaching intermediates.

The decay rate of the dichroism on a time scale of microseconds follows a simple exponential law, and is retarded when the viscosity is increased by allowing sucrose or glycerol to diffuse into the retina. The process therefore bears the mark of a diffusion-controlled rotational relaxation. Taking the rhodopsin to be a 45 Å sphere, the viscosity of its medium can be calculated from the rotational relaxation time as about 2 poise, which is in the range of thin oils. It inescapably follows that the membrane medium is essentially fluid. The viscosity is similar to that inferred by Weber for the interior of micelles from fluorescence decay measurements on trapped dyes. It may not pass unnoticed in this regard that attempts to use fluorescence depolarization of dye-labelled proteins to assess their mobility in membranes are misconceived, being limited by the singlet lifetimes of the dyes to much shorter time scales. The rod membrane, having practically no cholesterol, is probably more mobile than most.

The rhodopsin then must evidently be constrained (judging from the dichroism in the other direction) to float "upright" in the membrane, and must be supposed to be amphiphilic, one half of the molecule seeking the lipid

medium, the other half the aqueous exterior. The mobility is such as could easily allow the protein to act as a diffusional ion-carrier. Cone notes that there is X-ray evidence for a change in the rod membrane on bleaching, which has been interpreted in terms of greater submersion of the rhodopsin. Certainly bleaching does induce a conformational change, and perhaps exposes hydrophobic parts of the molecule. If this were so, the bleached opsin might be permitted to tumble freely, instead of orienting along the rod axis. Cone's conjecture is that the opsin might then become a calcium carrier because it is known that small changes in calcium concentration elicit a substantial change in sodium permeability of the rods. In this way the amplification, implicit by the measurable response of the rod to the arrival of a single photon, could be explained. The lessons of these results in relation to membrane structure and transport in general will not be lost on workers in the field.—From our Molecular Biology Correspondent.



FEW elementary reactions in the profuse canon of chemical kinetics have such a time-honoured respectability as this equation, with its associations going back to Bodenstein and beyond. Few would serve as better examples of the uneasy gulf between what one might pretend to know and what one honestly does know about the detailed dynamics of the collision process so confidently symbolized in the letters of the equation.

A monumental article from the Harvard chemistry department has now brought atom-molecule reactions of this type into the realm of what might reasonably be called high-precision reactive scattering studies. J. D. McDonald, P. R. LeBreton, Y. T. Lee and D. R. Herschbach (*J. Chem. Phys.*, **56**, 769; 1972) present well resolved data for the scattering of crossed beams of D atoms with the five halogen molecules:  $\text{Cl}_2$ ,  $\text{Br}_2$ ,  $\text{I}_2$ ,  $\text{ICl}$  and  $\text{IBr}$ . Angular scattering profiles are mapped, product velocity distributions are analysed and energy-disposal is accounted for, all at previously unheard-of signal/noise ratio.

Those chemists who have only passing acquaintance with the trials and tribulations of chemical molecular-beam techniques in the past fifteen or so years may be forgiven for not appreciating the full scale of the experimental *tour de force* which has made these results possible. More than any so far, they mark the undisputed arrival of the mass-spectrometer detector-analyser and the altogether timely end of what one writer has called the "alkali age"—the period when little could be detected at useful signal/noise beyond sodium and potassium beams the chemistry of which was, to say the least, unrepresentative of more normally interesting gas reactions.

At risk of sounding like a motoring correspondent I shall quote a few performance figures, which should at least deter anybody from rushing into the field with a raw research student and a little amateur glassblowing. The reaction chamber and beam sources were pumped differentially by batteries of ion and diffusion pumps up to 10 inch diameter; beams of halogen molecules and

D atoms, the first from a Laval nozzle, the second from a 2,800° K furnace, were collimated to 5° and crossed in the scattering chamber at no worse than  $10^{-6}$  torr background. The rotatable time-of-flight detector worked at 0.1 per cent ionization efficiency and could be pumped to better than  $10^{-14}$  background halogen pressures, the whole arrangement being coupled to a PDP8 computer for multi-channel analysis of the scattered products. Deuterium was used rather than hydrogen to give a ten-fold improvement in detector background noise. With all this the quality of the results approaches that previously obtained only in charged particle systems, with the proviso, that is, that the beams were not velocity-selected. Even at Harvard one cannot have everything. This, however, proves to be a relatively minor limitation to the experiment.

The choice of a reaction of a very light atom with a heavy molecule is an inspired one for, experimental advantages apart, this simplifies the dynamics to the point where there is a virtually direct perception of the relationship between scattering angle and the steric features of the three-atom potential-energy surface, and offsets most of the disadvantages of having a thermal spread of D atoms. From their results Herschbach and his team construct an intimate picture of the reaction dynamics somewhat as follows: the D atom, light and swift, steals up on the ponderous and unsuspecting halogen molecule until, almost at the instant of contact, it triggers a sudden electron rearrangement to a repulsive surface which promptly blows the products DX and Y apart in directions hardly different from the X-Y orientation at the start of interaction. The centre of gravity remains virtually fixed in the XY bond until decomposition; neither rotation nor vibration nor the momentum of the D atom plays a significant part and the scattered profile is virtually a record of the inclinations of the X-Y molecules at the initiation of successful reaction.

Now it has long been a received idea that reactions of the type  $\text{H} + \text{XY}$  should proceed through a strictly linear collision complex  $\text{H-X-Y}$ . (Only the most unkind would suggest that this might not be unconnected with the ease of drawing the traditional contour-map representation of the potential-energy surface for this case!) One of the first surprises in the data of the Harvard group is that hydrogen-halogen complexes are more often bent than straight—although  $\text{H-Cl-Cl}$  conforms with expectations,  $\text{H-I-I}$  seems to have an angle of some 100°,  $\text{H-Br-Br}$  is probably intermediate and, with the mixed halogens  $\text{ICl}$  and  $\text{IBr}$ , the lighter atom is extracted regardless of exothermicity, and determines the preferred angle. Roughly, then, the direction of the entrance valley into the energy-surface varies in a way correlated with the electronegativity of the middle atom.

Insofar as the  $\text{D-Cl-Cl}$  complex, at least, is linear, it is heartwarming to find that the results fit quite well with the semi-empirical energy-surface invented nearly forty years ago by Eyring and Kimball. The bent configurations require a more complex explanation, though, as the authors suggest, it may be sufficient to invoke the fact that the increased electronegativity of the end atom goes with a diminished electron density at the centre atom, an enhanced p-character of its orbitals and hence the bent geometry. All this points to some subtle topography around even three-atom potential-energy surfaces.

Many are the examples where considerable virtuosity in experimental technique has produced no very impres-



sive molehill of knowledge. What distinguishes this study is not only that the results are completely new and compelling in themselves but also that they have a healthy tendency to suggest more new problems than old ones solved. For the twenty years or so since kineticists began to admit that working out mechanistic crossword puzzles was not quite good enough, it has been fashionable to justify the most mundane rate measurements as a high-flown search for the real truth about potential-energy surfaces and the dynamics that takes place on them. For a long time this rang decidedly hollow; but with studies like the Harvard one the point is beginning to be made—one may indeed learn much about the topography of P-E surfaces for unstable systems which is not accessible by spectroscopy or other types of experimental probe.

One of the first messages to come through is almost a truism, though none the less worth stating: contrary to many preconceptions, there is no law that requires potential-energy surfaces to be any less individual, any more regular than the glorious vagaries of chemistry that they underlie.—From our Molecular Physics Correspondent.

## PLATE TECTONICS

### Finding Old Slabs

from our Geophysics Correspondent

WHEN a lithospheric plate, perhaps 50 km thick, starts its remarkable plunge into the Upper Mantle beneath an island arc, its identity gradually gets lost. It has been formed many million years before at a mid-ocean ridge by some process of fractionation, has travelled laterally as a sort of rigid boundary layer and now finds itself overridden by another plate and being transported downward at a dip of 40° to 50° into material which is several hundred degrees hotter. This process is occurring along about 40,000 km of plate boundary at the moment, and with a typical consumption velocity of 5 cm per year; 100 km<sup>3</sup> of plate is returned every year. If similar processes had persisted for all the  $4.5 \times 10^9$  years of the Earth's life (there is little evidence either way), the whole of the oceanic plate of the world could have been created and returned tens of times. Is there any way of seeking out some of this material, the oldest of which has possibly seen service as a plate more than once?

The past few million years worth of plate still manifests itself by earthquake activity within it which persists down to a depth of no more than 700 km. Studies of the deepest earthquakes show

that the slab at this depth is under compression from above within its plane, and it has been suggested that the slab, which after several million years is beginning to acquire the ambient temperature, is incapable of penetrating further into the mantle and may spread itself out like a carpet at this depth. Seismology seems the most likely way of finding this material, provided it has not been perfectly assimilated, but even though the slabs probably have a different chemical composition from the rest of the upper mantle because of their mode of formation, it is hard to imagine that the seismic velocity contrast amounts to more than 1 per cent at depth. Seismology is becoming increasingly capable of identifying heterogeneities, but the search for old slabs is likely to be arduous and the evidence is less than clear-cut in view of the other more pronounced heterogeneities already known to exist and overwrite the data.

Perhaps the most hopeful place to look for palaeoslabs is near recently inactive boundaries where extrapolation into the past strongly predicts that a region of consumption once existed. Seismicity still helps in some areas where, although the boundary is now no longer consuming, the deeper slab still apparently has not reached equilibrium. For instance, isolated pockets of deep seismicity in Burma, Hindu Kush and Romania seem to be testimony of busier times 20 million years ago when the whole southern edge of Eurasia was actively absorbing the Tethyan ocean. In 1954 a large earthquake 630 km below Spain in a historically aseismic zone was a singular reminder that seismicity is not an absolute term and some equilibrating processes in the Earth, arising from surface movements that ended tens of millions of years ago, must now be running very slowly. Is there any way, without waiting for a deep earthquake, that the palaeoslabs can be easily identified?

A report by McKenzie and Julian (*Bull. Geol. Soc. Amer.*, **82**, 3519; 1971) indicates a possibly powerful technique. The seismic velocity contrast between a fairly recently underthrust slab and the adjacent upper mantle can run as high as 5 to 10 per cent, particularly when the slab passes through the low-velocity zone 100–200 km beneath the Earth's surface. The slab, being colder than its surroundings, has the higher velocity. One of the most striking pieces of evidence in the emergence of plate tectonics was the demonstration by seismologists at the Lamont-Doherty Geological Observatory that elastic waves from deep earthquakes in the Tonga region that had travelled up the slab to stations in Tonga arrived 2 or 3 seconds earlier than waves that had

travelled the same distance in the opposite direction.

McKenzie and Julian concern themselves with a slab which has no deep seismicity to it whatever and use travel times of elastic waves from a shallow earthquake on top of the slab to teleseismic stations to show an apparent distortion of seismic velocity. It has been proposed that the Pacific seafloor is either still under-riding the North American continent or has only recently ceased to do so. This activity would occur beneath the Oregon, Washington and North California coast, the slab being created by an ocean ridge that exists a few hundred kilometres off shore. There is some seismicity along the coast line, none is deep, there is no sign of a present trench off the coast line but some seismic reflexion is evidence for under-thrusting and recent volcanic activity in the Cascades region. Evidence is thus mildly in favour of a slab under the region.

The technique used is known as the "residual sphere" method. Seismic residuals, the difference between the observed and theoretical travel time for a ray between an earthquake and a seismic station, are plotted on a notational sphere surrounding the earthquake. The position on the sphere corresponds to the emergence angle of the ray to get to the station in question. A large earthquake in Puget Sound, Washington, is considered and shows a band of negative residuals dipping on the sphere in an easterly direction at about 50°. This implies that somewhere between source and the stations reporting negative residuals is a region of higher than average velocity. A slab beneath Washington is an obvious explanation, but it is desirable to establish that it is a unique explanation. This is done by considering the residual sphere of an earthquake several hundred kilometres away on the Queen Charlotte Islands fracture zone, beneath which a slab would not be expected. The band of negative residuals has disappeared, which is strong evidence for the existence of a high velocity slab beneath the Cascades.

It is presumably going to be possible to outline other defunct slabs. The whole of Tethys might still leave some signs in seismic velocity perturbations. A much longer shot would be the very old slab that disappeared when Siberia and Europe collided to form the Urals. Travel time anomalies seem to be about a factor of two or three above the random "noise" for currently descending slabs. Searching for older slabs may call for much more detailed work. It will probably also call for an inverse approach in which a station rather than an earthquake in the appropriate region is studied for anisotropy of travel time from distant events.

# Fossil Lungfish from Australia

from a Correspondent

LUNGFISHES (Dipnoi), because of their close similarity in form to certain living amphibians, have always enjoyed a special place in the study of vertebrate evolution. The most primitive surviving lungfish, *Neoceratodus forsteri*, is found in Australia, and there is a certain piquancy in that one of the oldest known lungfishes, *Dipnorhynchus sussmilchi*, comes from the marine, early Devonian of the same continent. The structure and relationships of this Devonian lungfish are the subjects of a recent article by K. S. Thomson and K. S. W. Campbell (*Bull. Peabody Mus. Nat. Hist.*, **38**; 1971).

The skull roof of this primitive dipnoan seems essentially to have consisted of a mosaic of bones, and this is believed by some authors (for example, Westoll) to approach the condition in primitive bony fish. Thomson and Campbell re-evaluate the dermal bone patterns in Dipnoi, having assumed that *Dipnorhynchus* shows the most primitive pattern of dermal roofing bones. Why they make this assumption is not made clear, but it is presumably because of the age of the specimens. This is perhaps a dangerous presumption when the sparse and spotty fossil record of lungfishes is considered and it does not accord easily with some of the discussion in the last part of the article.

Using their model of the primitive condition (*Dipnorhynchus*), Thomson and Campbell discuss the pattern in other lungfishes in which there are fewer bones, assuming loss ("space capture")

and/or fusion. One may well question, however, whether they have succeeded in their professed aim that "an integral sequential system of analysis should replace the current *ad hoc* approach to the identification of bones. That is, using a given bone, as a primary reference point, other bones should be defined in sequence; each definition must be cast only in terms of entities defined earlier in the sequence" (see, for example, their Fig. 2). Thomson and Campbell make no homologies with the roofing bone patterns seen in crossopterygians or actinopterygians, but in an admirably clear account of the lower jaw they reject analysis based on a "primitive" mosaic of bones and draw direct topographic homologies with rhipidistians.

In respect of the brain case *Dipnorhynchus* and other fossil dipnoans can be closely compared with *Neoceratodus*. There are, however, some unexpected features in the otic region of *Dipnorhynchus*. According to Thomson and Campbell a sulcus on the outer face of the neurocranium in front of the supposed foramen for the vagus nerve is interpreted as the lateral occipital fissure and the vagus itself seems to have issued from the labyrinth. Alternatively, some workers might reinterpret the vagus as the glossopharyngeal (so that this nerve has its normal relationships) and the lateral occipital fissure as representing the existing back end of the specimen, the occiput having fallen away.

Thomson and Campbell return to the problem of the dermal roofing bone pattern in the discussion, because the relationships of dipnoans depend primarily on the interpretation of these bones. The case for a close relationship between the lungfishes and crossopterygians is argued and this is valuable, but few ichthyologists will regard this as the last word on the subject. In comparison with crossopterygians, Thomson and Campbell have to assume that the skull roof of *Dipnorhynchus* is derived from ancestors with fewer bones; this is a stumbling block because the subsequent evolution of lungfishes is generally agreed to involve the loss of bones and the acquisition of patterns not unlike those of other groups of fishes. Thomson and Campbell's view of lungfish evolution involves the acceptance of an abrupt and unexplained change in the direction of evolution.

In Thomson and Campbell's final analysis the new evidence presented on the dermal bone pattern of *Dipnorhynchus* could equally be taken to support the conclusion that the Dipnoi are

no more closely related to crossopterygians than to actinopterygians, but most workers will agree with the authors that the closest relatives of lungfishes are to be found among bony fishes.

## PROTEIN SYNTHESIS

### Eliminating Mistakes

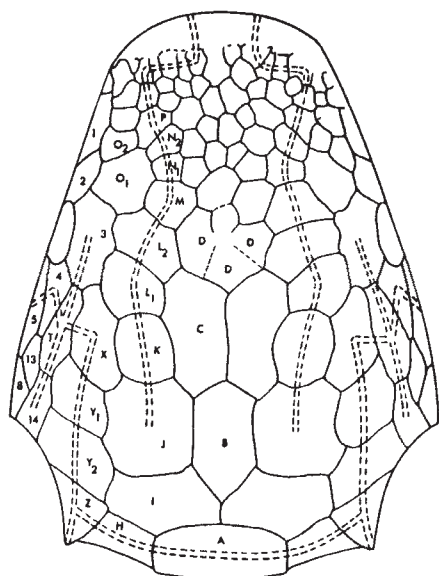
from our Molecular Genetics Correspondent

EACH of the stages of protein synthesis seems to take place with a remarkable degree of fidelity and even *in vitro* systems in which the various components of the synthetic apparatus are derived from different organisms can make recognizable proteins. Aminoacyl-tRNA synthetases place amino-acids on only the appropriate transfer RNAs and the action of the ribosome ensures that transfer RNAs make few mistakes in recognizing their codons on messengers. In spite of the precautions which seem to be built into the synthetic apparatus, however, it seems that *Escherichia coli* has a special system which is responsible for ensuring that any proteins bearing an excessive number of errors are destroyed.

Gross mistakes can result when a nonsense mutant causes a premature termination of protein synthesis so that a protein fragment much shorter than usual is released into the cell. Mutant proteins of this nature, such as the lactose repressor or the  $\beta$ -galactosidase protein coded by the *z* gene of the lactose operon, have a much shorter half-life than their full-sized counterparts. Most proteins in *E. coli* are stable, but some system seems to exist which degrades at least these particular mutants (see *Nature*, **228**, 1138; 1970). Goldberg has now shown (*Proc. US Nat. Acad. Sci.*, **69**, 422; 1972) that some such mechanism operates on many mutant proteins and that it can also detect and degrade more subtle mistakes than prematurely terminated proteins.

The general activity of the degradative system was revealed by growing *E. coli* cells in the presence of puromycin; this antibiotic bears a resemblance to aminoacyl-tRNA and so is able to enter the ribosome in place of it and to react with a nascent protein to set free polypeptidyl-puromycin. In this way it causes a general premature termination of protein synthesis. Goldberg finds that the proteins made in the presence of the drug are degraded to free amino-acids much faster than are normal proteins.

As well as recognizing differences in size, however, differences in amino-acid composition also seem to provide grounds for degradation. The *ram* (ribosomal ambiguity) mutant of the *E. coli* ribosome causes mistakes to be made in the recognition of codons on



Thomson and Campbell's restoration of the skull roof of *Dipnorhynchus sussmilchi* (from *Bull. Peabody Mus. Nat. Hist.*, **38**; 1971).



mRNA by anticodons on tRNA (one consequence of which is the suppression of nonsense codons). In *E. coli* cells carrying the *ram* gene, seven per cent of the cellular protein was degraded within 120 minutes, whereas in wild type cells only two per cent of the proteins was degraded in this time. Similarly increased rates of degradation were observed in strains of *E. coli* carrying mis-sense suppressor tRNAs, which only mistranslate the codons responded to by one tRNA molecule. Yet another test was to show that proteins containing analogues of amino-acids were much less stable than their wild type counterparts containing the proper amino-acids.

What is the basis for the action of the degradation system? Its general availability for prematurely terminated or mis-sequenced proteins implies that all normal *E. coli* proteins must share certain common features which mark them as inviolate from the action of whatever enzymes constitute the system. Deviations too far from these norms would make the proteins susceptible to proteolysis; small extents of mutation would presumably not matter. The common features are presumably concerned with the tertiary conformation of the protein, although this does raise the question of why incomplete proteins still under synthesis are not degraded; perhaps they are protected by the ribosome. One important experiment would be to detect mutants in the degradation system, but it is difficult to think of suitable selective tests. A particularly intriguing question is whether similar systems in other bacteria would recognize as normal similar features of tertiary structure, or whether each bacterial species has its own standards.

## BACTERIA

### Slimmed Down Phage

from our Cell Biology Correspondent

ON occasion and perhaps more often than might be anticipated, bacteria can, it seems, turn the tables on a virulent bacteriophage and render it into a bacteriocin, a defective phage particle unable even at very high multiplicities of infection to lyse its host cell but which can adsorb to and kill other sensitive bacteria. The colicins, bacteriocins of strains of *Escherichia coli*, needless to say, have been more extensively studied than their counterparts in the more arcane bacterial genera, but fortunately not everybody is wedded to *E. coli*. Recently, for example, in the *Journal of Virology* (9, 160; 1972) Lotz and Mayer have described an exemplary investigation of a bacteriocin produced by a strain of *Rhizobium lupini* isolated from the root nodules of a lupin. Al-

though lupins are not of very great economic importance, other legumes which harbour species of *Rhizobium* are extremely important crop plants and anything that furthers knowledge and understanding of bacteria of the genus *Rhizobium* is valuable.

It turns out that one of the strains of *Rhizobium lupini*, known as 16-3, produces a substance of high molecular weight that not only inhibits the growth of a second strain, designated 16-2, but also affects the replication of a lytic phage in strain 16-2. The bacteriocin, when added early during the latent period of the phage in 16-2 cells, inhibits their lysis whereas when added late in the latent period it accelerates lysis. Under the electron microscope samples of the bacteriocin can be seen to contain large numbers of particles which, with a sheath and core structure terminated by a base plate and spikes, closely resemble the tails of, for example, T-even coliphages.

When sucrose gradients of the bacteriocin preparations are assayed for bactericidal activity, the single peak of this activity corresponds with a small optical density peak and material in this fraction is seen in the electron microscope to consist exclusively of phage tail structures. As anticipated these tails adsorb to sensitive 16-2 strain cells and the sheath contracts on adsorption. Because the bactericidal activity resists

inactivation by doses of ultraviolet radiation as high as 6,000 ergs/mm<sup>2</sup> it seems unlikely that the phage tails contain any nucleic acid and it is much more likely that the tails kill sensitive cells either by injecting lytic enzymes or by acting at the cell surface. But in any event it seems clear that the bacteriocin of *R. lupini* strain 16-3 is an incomplete phage particle.

Delk and Dekker (*J. Mol. Biol.*, **64**, 287; 1972) reach what is essentially an identical conclusion about the structure and origin of the so-called rhabidosomes (rod shaped particles) liberated by an even less familiar bacterium, *Saprospira grandis*, a marine flexibacterium. The elegant electron micrographs of these authors clearly reveal that rhabidosomes are in essence two overlapping hollow cylinders. The core cylinder is some 4,300 Å long and 90 Å in diameter and the sheath cylinder is 2,000 Å long, 250 Å in diameter and has a channel 110 Å in diameter. Amino-acid analyses of core and sheath prove that the two components are made of distinct protein subunits and there is no nucleic acid associated with the rhabidosome. Delk and Dekker attribute earlier claims that these structures contain methylated RNA to contamination of the rhabidosomes with cellular RNA.

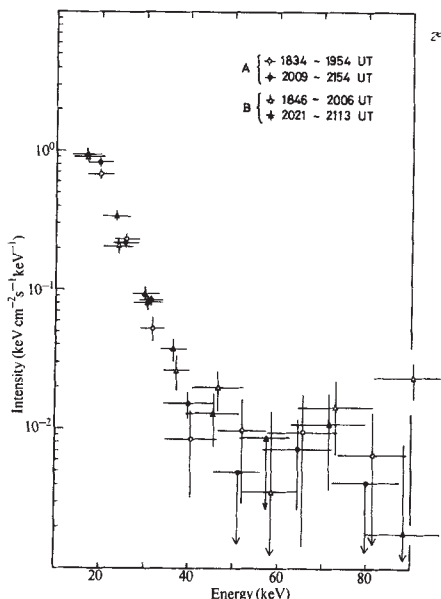
Both the chemical composition and the structure of the core and sheath are so reminiscent of bacteriophage tails

## Sco X-1 at Optical and X-ray Frequencies

It is vital for an understanding of the X-ray source Sco X-1 that it should be determined unambiguously whether or not the optical activity of the source is related to the X-ray variations. Some recent work has found an inverse correlation between optical luminosity and intensity of hard X-rays (T. Kitamura *et al.*, *Astrophys. Space Sci.*, **12**, 378; 1971); other reports suggest a coincidence between optical and X-ray activity (H. S. Hudson *et al.*, *Astrophys. J. Lett.*, **159**, L51; 1970). Part of the problem of resolving this discrepancy, of course, lies in the difficulty of timing balloon or rocket flights to coincide with optical observations.

In next Monday's *Nature Physical Science* (March 27), M. Matsuoka *et al.* report further simultaneous observations of this source at optical and X-ray frequencies, and this work supports the evidence for a positive correlation between optical and thermal X-ray emissions. It is also interesting that the X-ray spectra of Sco X-1 show a possible non-thermal component at energies above 50 keV (see figure) which may be enhanced when the source is optically bright. Although this work goes some way towards ex-

plaining previous contradictory results, further observations will be required before the nature of the energy generation mechanism becomes clear.



Spectrum of Sco X-1. Circles and triangles represent intensities obtained with two different counters on the same rocket flight on May 1, 1971.

that there seems little doubt that rhabdosomes are vestiges of once complete bacteriophages and that the genomes which specify these particles are now stably associated with the genome of the host cell. Among bacteria the persistence of such vestigial virus particles and their genomes as symbionts seems to be the rule rather than the exception.

## CANCER RESEARCH

### Tumour Vascularity

from a Correspondent

PROFESSOR L. F. LAMERTON (Institute of Cancer Research), introducing the topic of tumour blood supply and metastasis at the ninth meeting in the principles of experimental and clinical oncology series in the British Museum (Natural History) on March 6, pointed out that the type, arrangement and morphology of blood supply are important elements in the heterogeneity of tumours, both between tumours of the same type in different individuals and within single tumours. He also suggested that blood supply can be a chief factor in determining the likelihood of metastases or the outcome of chemotherapy or radiotherapy. These themes were developed by the other speakers, Dr R. H. Thomlinson (Radiobiology Unit, Royal Postgraduate Medical School) and Dr M. J. Peckham (Institute of Cancer Research).

Dr Thomlinson pointed out that in different tumours the clonogenic (or proliferating) cells are found at various distances away from blood vessels, and this variation greatly affects the physiological condition of the cells and their susceptibility to attack, particularly by irradiation. In deeper regions of the tumour capillaries are likely to be attenuated and are possibly inefficient carriers of blood. There is considerable likelihood of shunts developing by anastomosis of blood vessels in the more superficial regions of the tumour. Overall blood flow through tumours tends to be slow and this can possibly be attributed to venous congestion, perhaps arising from pressure within the tumour. All too little is known about lymphatic drainage of tumours.

In discussion it came up that skin homografts without a lymphatic drainage but with an otherwise normal blood supply are not rejected. It was agreed that if the lymphatic drainage of tumours is degenerate (Dr R. J. Goldacre, Institute of Cancer Research) this could be an important factor in limiting immunological attack on a tumour. In relation to radiotherapy, in which it is known that the sensitivity of tumour cells to ionizing radiations is affected

by oxygen concentration, Dr Thomlinson felt that it was important to get some idea of the pattern of re-oxygenation as the tumour mass is reduced, but it is very difficult to obtain this sort of information.

Dr Peckham described experimental studies which show that failure of development of a blood supply can limit the growth of a tumour. Nevertheless in most situations it seems that tumours have a considerable capacity to induce capillary growth, even to the extent of producing angiogenetic factors. Antibodies against these materials have been used to attempt to limit metastatic spread of tumours but so far with no success. Dr Peckham also considered the various claims that have been made that tumour cell masses are surrounded by a fibrin lattice and that this is an important factor in either tumour growth or the tendency to metastasize. He spoke of evidence that injection of fibrinolysin can reduce the incidence of metastases but said that there are various and partly conflicting results. In similar experiments it has been shown that warfarin increases the prothrombin time to two to three times normal and there can also be a reduced incidence of metastases. Here, however, it is difficult to exclude a direct effect of the warfarin on populations of tumour cells.

The meeting revealed that although tumour vascularity and the relationship of the tumour to the connective tissue materials in which it grew are topics of considerable import the complexities of the situation have tended to defy useful analysis.

## BIOPOLYMERS

### The End of the Tether

from our Molecular Biology Correspondent  
THE notion of tethering biochemically interesting molecules, such as enzymes, to insoluble matrices by way of covalent bonds can scarcely be seen as a new and revolutionary insight. The first successful attempts at such a strategy with a view to controlling the exposure of substrates to enzymes, and for isolating biologically active species by affinity methods, were indeed described many years ago, but it is only really in the past year or two that this general approach seems to have become lodged in the collective subconscious of the biochemical cohorts. In this time it has without doubt brought about a minor revolution in biochemical practice, and the scope of the applications is still widening, partly at least under the stimulus of various technical improvements. Some interesting examples are to be found in the current literature.

One area that has been completely transformed by the matrix attachment approach is that of peptide synthesis, the efficiency of which has now reached a level that would have been unthinkable in the days when the product after each addition had to be recovered from solution. The efficiency of the method is limited by the extent to which any addition cycle falls short of complete reaction, and this allows truncated or incorrect chains to be formed in appreciable amounts. Bayer *et al.* (*J. Amer. Chem. Soc.*, **94**, 265; 1972)

### Alternation of DNA Conformations

IN next Wednesday's *Nature New Biology* (March 29) Pilet and Brahms report a reinvestigation of the B $\rightleftharpoons$ A transition of DNA, in relation to its base composition. The B form was first recognized at high humidities, and it is presumed to represent the state of DNA in solution. The bases are tilted relative to the helix axis, whereas in the A form the bases lie perpendicular to the axis. The precise humidity at which the A form appears depends critically on the salt concentration. At lower humidities denaturation occurs and the ordered structure is lost. Because of the difference in the tilt of the bases, and accompanying changes in the orientation of the phosphate groups, it is possible to distinguish the A and B forms by measurements of infrared dichroism of oriented films.

Pilet and Brahms have observed the B $\rightleftharpoons$ A transition by this means and find that in typical DNA of relatively high (G+C) content, the change occurs

within a very narrow humidity range. They find, however, that the lower the (G+C) content the more reluctant the A form is to appear. In the extreme case of crab satellite DNA, which contains less than 5 per cent of (G+C) and is essentially an alternating polymer of A and T, the A form does not appear at all, the B structure giving place directly to the denatured state, the unfolding beginning below 90 per cent relative humidity.

Pilet and Brahms have constructed a phase diagram indicating the ranges of stability of the A, B and denatured forms of DNA as a function of base composition and relative humidity; from this it seems that if the (G+C) content falls below about 35 per cent the zone of stability of the A form disappears. The possibility that inhomogeneous distribution of base composition along a DNA will give rise to an alternation of conformations within a single molecule must be considered.



have devised a neat method for rapid screening of the product after each cycle. It depends on the introduction of a trifluoroacetyl group as a substituent at the amino terminus of the peptide, and the observation that its  $^{19}\text{F}$  nuclear magnetic resonance spectrum is subject to very large chemical shifts, the magnitude of which is a function of the adjoining side chain. An examination of the trifluoroacetyl amino-acids shows that chemical shift values can be uniquely assigned to all of them. At each step of the synthesis, therefore, a small aliquot of the matrix bearing the peptide is removed, the peptide is hydrolysed off, reacted with trifluoroacetic anhydride, and its  $^{19}\text{F}$  n.m.r. spectrum gives forthwith a quantitative analysis of the relative end group concentrations. This seems to be an application of wide potential utility.

The choice of supports for the attachment of proteins is now quite wide, though agarose beads, activated with cyanogen bromide to react with primary amino groups of proteins, as developed by Porath and Axén, are not commonly used. In many cases, however, the activity of a bound molecule depends on the properties of the support, and the nature and length of the linking group, for these seem often to control the accessibility to reacting species. The preparation and use of two supporting media are described by Brümmer *et al.* (*Europ. J. Biochem.*, **25**, 129; 1972). One is carboxymethyl cellulose derivatized to give the highly reactive acid hydrazide; the other is a copolymer containing maleic anhydride, which will react directly with proteins in solution. With a variety of proteolytic enzymes active products are obtained, the activity being in general a good deal higher towards small than large (polymeric) substrates, presumably in virtue of restricted access. As has been observed in other cases, the thermal stabilities of the enzymes in the bound state tend to be higher than in solution, and autolysis will be expected to be minimized. The insoluble enzymes can be used repeatedly with little deterioration.

Another medium that has now been successfully used as a support (Filippusson, Hornby and McDonald, *FEBS Lett.*, **20**, 291; 1972) is nylon 66, which is a relatively hydrophobic material, containing primary amino groups. These are linked to the lysine groups of the protein—in this case urease and urate oxidase—by way of glutaraldehyde. The support may be in the form of a powder, which is packed into a column, or nylon tubes can be used, through which substrate is then percolated. Filippusson *et al.* report that a sample of urease coupled to a nylon tube was used over a period of four months for more than 400 urea assays.

Again the stability of the enzyme is clearly enhanced in the immobilized state. The future development of automated analysis systems may be linked to the use of such preparations.

Gyenge, Spiridonova and Bogdanov (*ibid.*, 209) have bound ribosomal proteins to agarose beads and have shown that they can selectively bind their corresponding ribosomal RNA, which suggests a means of isolating RNA fragments containing individual protein binding sites. Conversely RNA can be coupled to a matrix, and a variety of applications for the resulting material can be envisaged. A method based on the formation of a hydrazone between the periodate-oxidized 3'-terminal sugar and the derivatized agarose containing hydrazide groups is available. Robberson and Davidson (*Biochemistry*, **11**, 533; 1972) have shown that a high yield of coupling is achieved only if the negatively charged carboxyls in the matrix are blocked, an objective which they accomplish by a carbodiimide coupling reaction. Poly U coupled in this manner will catch poly A—a feature that may find its uses, for example, in isolating poly A-containing eukaryotic messengers. Ribosomal DNA sequences can also be trapped by ribosomal RNA bound to a column.

An article by Gawronski and Wold (*ibid.*, 442) shows the advantages that immobilization of one component can give in the study of binding processes. They have examined the interaction of ribonuclease S-peptide (the fragment that can be removed from the subtilisin-treated enzyme with reversible loss of activity) with the residual S-protein, by coupling the former to an agarose column. Binding is simply measured by the concentrations of S-protein left in the supernatant. The complex on the matrix is enzymatically active. Good binding data can be extracted, the association constants being, however, somewhat higher than those derived from solution measurements. The authors conjecture that the higher concentrations used in the latter might have introduced discrepancies due to self-association of S-protein.

A further article (*ibid.*, 449) attempts a more detailed thermodynamic study, but the system is evidently of some complexity, the van't Hoff plots being curved, except perhaps at low temperatures. Complications arising from self-association and other side reactions are inferred. At low temperatures the authors conclude (though this is at odds with earlier work) that the association is a primarily entropy-driven process.

## RNA Adaptors in DNA Replication

MODELS seeking to explain how DNA replicates semiconservatively abound in the literature of molecular biology and will no doubt continue to flourish until more pertinent experimental data have been accumulated. Few of the familiar models, however, carry the stamp of ingenious originality which marks what Brewin proposes in next Wednesday's *Nature New Biology* (March 29). Brewin postulates that a series of sixty-four adaptor RNA molecules, one for each codon, exist and that each carries its particular codon or DNA trinucleotide attached at the 3' end to the replication site in a way analogous to the transport of amino-acids by transfer RNAs during protein synthesis.

In fact it was apparently while pondering the evolution of the mechanism of protein synthesis directed by polynucleotides that Brewer began to wonder whether adaptor RNAs might be operative in DNA replication. He argues that before protein synthesis could have evolved a system of polynucleotide replication must have become established. Furthermore the transfer and ribosomal RNAs, called polynucleotide enzymes by Brewin, rudimentary forms of which must presumably have been required in even the most primitive protein synthesizing systems, most likely evolved from parts of the

machinery of polynucleotide replication.

Such arguments lead to a model of DNA replication in which RNA adaptor molecules become charged at their 3' ends by DNA codons the sequence of which is determined by an internal template, three bases of the adaptor RNA chain. Charged adaptor molecules can then deliver their DNA triplets to the nascent DNA chains while the internal template of the adaptor RNA, which specified the DNA codon being carried, base pairs with the complementary codon on the parental strand of the template.

As Brewin points out, the great attraction of this model is its ability to account for the specificity and mechanics of DNA replication at a stage in evolution at which proteins were inaccurately specified and comparatively undifferentiated. With the evolution of proteins the absolute need for RNA adaptor molecules in DNA replication has no doubt diminished and the ancestral mechanism of DNA replication has no doubt changed as a result of evolution, but it has probably not changed beyond all recognition. Indeed, Brewin not only suggests that several recent observations about the mechanism of DNA replication in bacteria are consistent with his model but also suggests simple experiments to test his speculations.

# The Significance of Glycosylated Proteins

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**It has been suggested that glycosylation is a means of labelling proteins for intracellular recognition and export. This hypothesis is rejected in favour of the idea that the attached carbohydrate determines the extracellular fate of the protein molecule.**

GLYCOPROTEINS are polypeptides containing a variable amount of covalently attached carbohydrate. They are widespread in the plant and animal kingdoms and have numerous diverse functions, for example, hormonal, structural, enzymatic, and in transport, lubrication and cellular adhesion (for recent reviews see refs. 1-3).

During the past decade the number of proteins known to contain covalently bound sugars has increased markedly and many proteins which were believed to be uniquely amino-acid in composition have now been found to occur in a glycosylated state. The universality and variability of saccharide structures encountered have impeded the development of a unifying hypothesis for why the cell should attach these residues to the parent protein. It is not surprising that there have been few attempts to justify the biological role of these sugars on glycoproteins. Eylar<sup>4</sup> suggested that the attachment of sugars to a protein was the chemical passport for export from the synthesizing cell, and, reciprocally, intracellularly confined proteins were non-glycosylated. The inclusion of the sugars represented a chemical recognition signal to enable a cellular sorting process to distinguish between those proteins destined for intracellular use and those whose fate was extracellular. So far this attractive hypothesis has received no significant endorsement or rebuttal. It is our aim in this article to reinvestigate the evidence on which Eylar's postulate rests and to discuss other possible physiological roles for the inclusion of sugars on protein structures.

The prerequisite in discussing Eylar's hypothesis is a clear distinction between that which is inside the cell and that which is outside. Eylar defined "extracellular" as that which is external to the plasma membrane. We propose that this be amended to include materials which are committed irrevocably to the extracellular milieu or are in continuity with it. This redefinition includes as outside the cell such areas classically intracellular as the cisternae of the endoplasmic reticulum, the inside of the Golgi sacs and vesicles and the interior of the lysosomes and phagosomes. The relationships of these organelles demonstrate that vesicles are formed by "pinching" of membranes. The contents of such a vesicle are there-

fore derived from the contents of the membranous system which generated it, for example, the formation of primary lysosomes from the mature face of the Golgi stack. Similarly, because these vesicles condense rather than ingest one another, the contents of such vesicles are continuous. On the other hand, the engulfing of a mitochondrion by a lysosome is consistent with the contents of these two membrane bounded organelles not being continuous. It is inherent in this distinction that an exportable protein traverses only one membrane interface, the endoplasmic reticulum. The position regarding the membranous proteins is ambivalent, though it seems reasonable to suggest that the intracellular continuum is governed by free diffusion of the components and therefore the membranous interface can be described as extracellular.

To appreciate this definition it is important to review briefly the principal cellular events leading to the synthesis of a secreted protein. Intracellular proteins are synthesized by free ribosomes while it has been shown in several tissues that the polypeptide of the exported protein is assembled on membrane bound ribosomes<sup>5-9</sup>. The nascent polypeptide chain is immediately transported through the endoplasmic reticulum into the cisternal space<sup>5,6,9</sup> at a locus very close to the ribosomal point of attachment<sup>10</sup>. In some cases the primary glycosylation is performed either as the polypeptide passes through the membrane or immediately thereafter<sup>11,12</sup>, although with other cell types studies with puromycin suggest that a close interdependence between protein synthesis and glycosylation may not be universal<sup>1</sup>. The synthesis of the bond between carbohydrate and polypeptide depends on the recognition of a particular sequence of amino-acids, for example, Ser-Gly-Gly for glycosaminoglycan synthesis<sup>13</sup> or Asn-X-Thr/Ser for most plasma glycoproteins in which N-acetylglucosamine is linked by a  $\beta$ -glycosidic bond to the amido group of asparagine<sup>1,2</sup>. On the basis of their work on ribonuclease B, Jackson and Hirs<sup>14</sup> proposed that the nature of the intermediate amino-acid, X, dictated the type of oligosaccharide unit synthesized. Although other work<sup>15</sup> has contradicted this theory, at present there is no other proposal regarding the coding for the different types of carbohydrate unit attached to a particular polypeptide. Subsequent glycosylations are catalysed by a series of membrane bound enzymes, the final organization of the monosaccharides within the chains being dictated by the specificities of each enzyme for the nucleotide sugar and the acceptor<sup>16</sup>. This arrangement whereby the product of one glycosyltransferase becomes the substrate for the next similar reaction has led Roseman to propose that the entire system constitutes a multi-enzyme complex<sup>16</sup>. Subcellular fractionation and autoradiography have demonstrated the cytological relationships of the glycoside addition. The initial glycosylations, for example, the formation of the core region of the



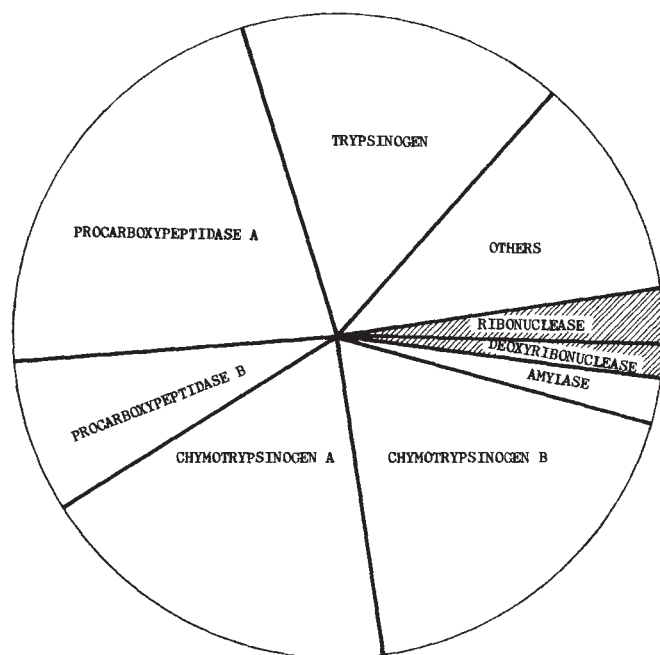


Fig. 1 Quantitative distribution of proteins in bovine pancreatic juice<sup>28</sup>. The shaded area represents those that are known to be glycoproteins.

plasma glycoproteins, occur on the rough endoplasmic reticulum while the peripheral additions are made during the passage of the protein through the smooth membranes and within the Golgi vesicle<sup>12,17-20</sup>. The terminal sialic acid and fucose residues are added very late in the temporal scheme of the synthetic process, possibly immediately prior to secretion<sup>19,20</sup>. In addition to the synthetic capacity of the Golgi apparatus, this organelle concentrates the intravesicular materials<sup>21</sup>. The vesicles at the mature surface of the Golgi stack give rise to either lysosomes or secretory vesicles. The secretory process is probably a reversal of pinocytosis involving the fusion of the vesicles with the plasma membrane<sup>18</sup>. It is significant that albumin, a non-glycosylated extracellular protein, is assembled and secreted by the same cytological route<sup>22</sup>. Furthermore, the evidence does not support a contention that albumin is a glycoprotein which has the carbohydrate removed before secretion. Thus present knowledge furnishes no evidence for the existence of different biosynthetic routes for glycoproteins and non-glycoproteins.

The membranous proteins of the endoplasmic reticulum, Golgi, plasma membrane and also probably the lysosomes are assembled in similar fashion from the membrane bound ribosomes except that they are incorporated into the membrane rather than liberated into the cisternal space<sup>16,21-24</sup>. In immunoglobulin biosynthesis the close similarity of these pathways is emphasized by the fact that the same protein may be incorporated into the plasma membrane as is secreted in soluble form.

Although most extracellular proteins are synthesized by the route described, or a minor variation of it, collagen biosynthesis is a notable exception. The polypeptide is not secreted by way of the Golgi system but passes directly to the plasma membrane where the carbohydrate residues are added as it is extruded<sup>25</sup>.

One problem which has been receiving recent attention is that the glycosyltransferases are located in an extracellular position while the nucleotide sugar substrates are synthesized in the cytosol<sup>26</sup>. The evidence for the translocation of the sugar moiety across the membrane favours the participation of a polyphenol lipid carrier<sup>27</sup> as originally proposed in the biosynthesis of bacterial cell wall peptidoglycans.

It is apparent from this description of the biosynthetic process that the signal for export resides in the messenger RNA since the discrimination of the messenger RNA for a membrane bound ribosome is the commitment for the synthesis of an exportable protein. The decision for export has been made before the addition of carbohydrate and is *ab initio* genetically specified rather than indirectly determined through the synthesis of glycosylating enzymes. Inasmuch as the passport for export is determined before glycosylation rather than *vice versa*, this is one argument against Eylar's hypothesis.

A second area in which Eylar's hypothesis can be examined is in the review of the complete protein output of a few tissues which are clearly devoted to the synthesis of extracellular protein. The sole criterion in our selection of these examples was the availability of a complete analysis of the production of these tissues.

The best documented tissue is the exocrine pancreas. The protein composition is accurately known for the bovine species (Fig. 1) as is the rate of synthesis<sup>28</sup> (up to  $1 \text{ g h}^{-1}$ ). Only ribonuclease B<sup>29</sup> and deoxyribonuclease<sup>30</sup>, both relatively minor components, are known to be glycoproteins; the remainder do not have carbohydrate attached.

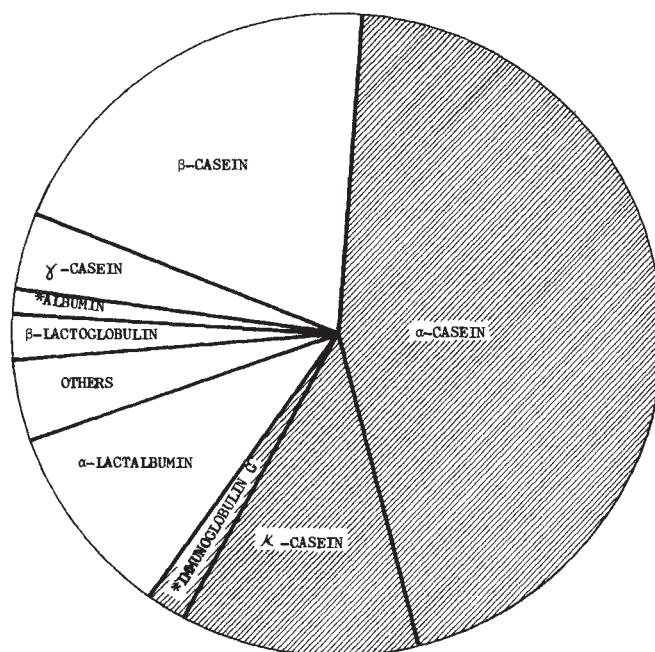


Fig. 2 Protein composition of mature cow milk from ref. 31. The shaded area represents those that are glycoproteins and the asterisks denote components derived from the blood.

Two secretions which differ from the exocrine pancreas in being nutritive rather than enzymatic are milk and egg white. The total analyses of these are shown in Figs. 2 and 3 for mature cow milk<sup>31</sup> and hen egg white<sup>32</sup> respectively. In milk the glycoproteins are  $\alpha$  and  $\kappa$ -caseins<sup>31,33</sup>, a minor variant of  $\beta$ -lactoglobulin<sup>31</sup> and one form of  $\alpha$ -lactalbumin<sup>34</sup>, while of the proteins present in egg white only lysozyme is not a glycoprotein<sup>33</sup>.

The liver is the primary site of plasma protein synthesis, the immunoglobulins being the only group not produced in this organ<sup>35,36</sup>. Table 1 shows the protein composition of human plasma<sup>35-37</sup>. Although the only plasma proteins not glycosylated are albumin<sup>36</sup>, prealbumin<sup>36</sup> and retinol-binding protein<sup>38</sup>, these represent 48% by weight of the total. Furthermore, when the rates of synthesis of some of these proteins are studied using an isolated perfused rat liver which gives a truer indication of the output of this organ, albumin figures prominently<sup>37</sup>.

The anterior pituitary is an example of an organ which secretes hormones. Six well authenticated polypeptide hormones are released, three of which are glycoproteins<sup>1</sup> (luteinizing hormone, follicle-stimulating hormone and thyrotropic hormone) and three of which are not<sup>39,40</sup> (growth hormone, adrenocorticotrophic hormone and prolactin).

**Table 1** Protein Composition of Human Plasma derived from Ref. 36

| Protein                      | Mean plasma concentration mg 100 ml. <sup>-1</sup> |
|------------------------------|----------------------------------------------------|
| <b>Non-glycoproteins</b>     |                                                    |
| Albumin                      | 4,000                                              |
| Prealbumin                   | 32                                                 |
| <b>Glycoproteins</b>         |                                                    |
| Immunoglobulins              | 1,690                                              |
| Fibrinogen                   | 400                                                |
| $\alpha_1$ Antitrypsin       | 350                                                |
| $\beta_1$ Lipoprotein        | 360                                                |
| $\alpha_1$ Lipoprotein       | 330                                                |
| $\alpha_2$ Macroglobulin     | 300                                                |
| Transferrin                  | 260                                                |
| $\alpha_2$ Lipoprotein       | 190                                                |
| Haptoglobin                  | 110                                                |
| Haemopexin                   | 90                                                 |
| $\alpha_1$ Acid glycoprotein | 88                                                 |
| Plasminogen                  | 75                                                 |
| B <sub>1c</sub> globulin     | 35                                                 |
| Caeruloplasmin               | 33                                                 |
| Others                       | 50                                                 |

Immunoglobulins are synthesized by the plasma cells and are apparently the only proteinaceous material secreted by this tissue. Although all immunoglobulins known so far possess carbohydrate, various myeloma lines secrete L-chains devoid of sugar residues<sup>9,41</sup>.

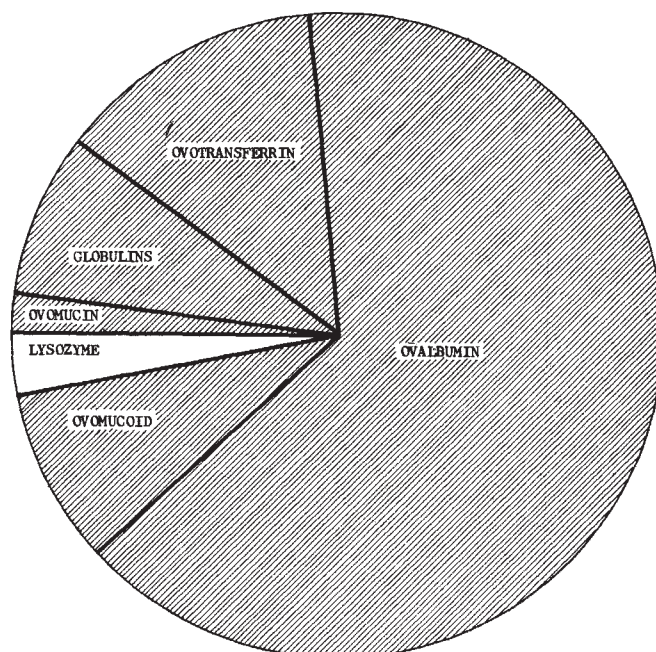
Fibroblasts synthesize and secrete collagen, probably tropoelastin and a spectrum of glycosaminoglycans. The extrusion of collagen through the plasma membrane does not, however, have an obligatory requirement for the addition of the disaccharide unit, for it has been reported that silk collagen of the sawfly is not glycosylated<sup>42</sup>. Similarly, porcine tropoelastin has been found without covalently bound carbohydrate<sup>43</sup>.

It is apparent from these examples that a significant and variable proportion of exported proteins are not glycosylated and it is difficult to reason why the secretion of some proteins should apparently require the concomitant presence of sugars and others not.

The converse argument of Eylar was that the intracellular proteins are not glycosylated. Even with the limitations imposed by the redefining of "intracellular" which excluded the glycoprotein lysosomal hydrolases<sup>21,44</sup> and the proteins of the endoplasmic reticula, Golgi, lysosomal and cell membranes<sup>1,45-47</sup>, there is a significant and growing number of glycoproteins found within the cell. These include  $\gamma$ -glutamyl transpeptidase<sup>48</sup>, aspartate aminotransferase (super  $\alpha$  form)<sup>49</sup>, the sialoglycoprotein between the inner and outer mitochondrial membranes<sup>50</sup> and proteins present in the mitochondrial<sup>51,52</sup> and nuclear membranes<sup>53</sup>.

Thus it seems that there is little secure ground left to defend the proposal that "the carbohydrate unit would serve as a passport which would promote the exit of the protein from the cell". If the original contention of Eylar has been shown to be unsound, we are left with the initial question—why are the sugar units on glycoproteins?

The mucins and glycosaminoglycans, which though not normally classified as glycoproteins do have sugars covalently linked to a polypeptide core, are examples of material where certain functions can be attributed to the sugar structures. Many of these contain charged groups such as carboxylate (uronic and sialic acid residues) or sulphate and these



**Fig. 3** Protein composition of chicken egg white from ref. 32. The shaded area denotes the components with covalently attached carbohydrate.

confer on the polymer marked polyelectrolyte behaviour which accords with their physiological role. But with a large number of glycoproteins the physicochemical properties imparted by the carbohydrate moiety do not apparently justify their inclusion on the polypeptide chain.

The application of inductive reasoning to why certain proteins need to be glycosylated leads to certain possibilities which will now be discussed. (a) Although the small polypeptide hormones are devoid of carbohydrate, the ranges of molecular weights observed for the two protein categories are very similar. Thus the size of the molecule has no bearing on whether it is a glycoprotein or a non-glycoprotein.

(b) Present information does not lead us to suspect that the synthesis and metabolism before secretion are influenced by the carbohydrate moiety. The anterior pituitary synthesizes adrenocorticotrophic hormone at the same rate as thyrotrophic hormone, the liver exhibits similar synthetic rates for several plasma proteins which differ in carbohydrate content<sup>35</sup> and ovalbumin is elaborated at the same rate as the other proteins of the oviduct<sup>8</sup>. Many secretory cells store material in granules or vesicles before release. The presence of carbohydrate does not affect the rate of storage or the amount stored as can be seen from the rates of synthesis and cell contents of the proteins of the anterior pituitary or pancreas.

(c) The morphology of a cell does not seem to have any bearing on the type of protein synthesized. Nakane<sup>39</sup> has shown that it is impossible to distinguish between adrenocorticotrophic cells and thyrotrophic cells by their ultrastructural characteristics alone but requires the use of immunocytochemical methods.

(d) As mentioned earlier, the initial glycosylation which converts a protein into a glycoprotein probably depends on a particular sequence of amino-acids coded by the messenger RNA. It is possible to propose that whenever evolutionary mutations have generated this sequence in a polypeptide carbohydrate was covalently attached. In this manner many glycoproteins would be accidents of nature. Although this may be the case in the synthesis of the variable regions of immunoglobulins<sup>15,41</sup>, there are a number of documented primary structures of secreted proteins possessing the required coding which have not been glycosylated<sup>54-56</sup>. Furthermore, some of these proteins have been secreted from cell types capable of



glycosylating other polypeptides with the appropriate coding.

(e) On the basis of the embryological origins of tissues involved in secreting proteins and homologues in amino-acid sequences, Adelson<sup>57</sup> proposed an evolutionary hypothesis to account for extracellular proteins. He has suggested that this class of protein evolved by gene duplication, mutation and selection from the digestive enzymes of simpler forms of life. But although this hypothesis accounts for similarities in the extracellular proteins and the origin of the partial proteolytic digestion method of activation, it does not provide a clue to the function of the carbohydrate present on many of these proteins.

(f) Another theoretical proposal which we can suggest is that the glycoproteins which in terms of nature are ancient are vestiges of the evolutionary process. Thus the cell coat of microorganisms, which is predominantly carbohydrate in composition, contains a number of proteins located at the interface with the extracellular environment. Some of these proteins, which may have been hydrolytic enzymes, were released from their attachment to the cell carrying with them vestiges of the carbohydrate matrix. An example of this is the extracellular nuclease of *Micrococcus sodonensis* which contains 21% carbohydrate chemically and immunologically identical to the cell wall carbohydrate<sup>58</sup>. We are unable to refute this suggestion but consider it unlikely.

No apparent explanation results from these proposals as to why certain proteins are glycoproteins. If we have to ascribe a function for the carbohydrate moiety of the protein it is an important prerequisite that the complete biological function of the entire molecule must be understood. It may be possible then to examine whether the carbohydrate is important in the performance of all or part of this function. The term "complete biological function" is important. Thus, it is possible to remove most of the carbohydrate from certain glyco-enzymes without impairing the enzymatic function, for example, thrombin<sup>59</sup>, glucoamylase<sup>60</sup> and chloroperoxidase<sup>61</sup>. Similarly, carbohydrate residues can be removed from plasma glycoproteins without affecting transporting functions<sup>62,63</sup> or tertiary structure<sup>64,65</sup>. Nevertheless the doubt must still remain whether the complete biological function of a protein such as transferrin is to transport iron. In contrast there is a group of glycoproteins in which dramatic consequences attend the removal of even small saccharide units. Examples of this group are the carbohydrate-containing hormones of the anterior pituitary where modification of the carbohydrate portion abolishes the specific interaction of a hormone with the target organ<sup>1,33,66,67</sup> and the blood group specific glycoproteins<sup>35,68</sup>. This category may be larger if we consider those proteins which are activated by limited proteolysis; for example fibrinogen<sup>69</sup> which requires intact carbohydrate for clot formation and  $\kappa$ -casein<sup>33</sup> in which the liberation of a glycopeptide containing the majority of the carbohydrate destroys the micellar stabilizing properties of the protein. Another attribute of sugars is seen in the proposal that the disaccharide units attached to hydroxylysine residues in collagen may determine the packing arrangement of the fibrils<sup>70</sup>.

Caeruloplasmin and other plasma proteins with the apparent exception of transferrin are rapidly removed from the circulation by the parenchymal cells of the liver if a few sialic acid residues are removed<sup>71</sup>. The desialylation results in the penultimate galactose residue becoming terminal and this oligosaccharide unit is the determinant that combines with receptors in the plasma membrane. The catabolic process is completed in the lysosomes<sup>1</sup> presumably after pinocytosis of the membrane with the associated proteins bound to the receptors. If this desialylation process reflects the *in vivo* mechanism of turning over the plasma proteins, the rate of catabolism will be influenced by several factors such as the binding affinity of the various desialylated proteins to specific sites on the plasma membrane, the number of such sites and the rate of pinocytosis. The removal of perhaps less than 0.1% of the com-

ponents of the molecule has profoundly changed the pattern of response of the cell for the protein from one of non-recognition to one of positive recognition. The temporal control of this catabolic process must reside in the neuraminidase which removes this terminal residue. Therefore, modulations in the rate may be achieved by synthesizing the peripheral oligosaccharide chains with differing chain terminants, for example, N-acetyl-, N-glycolyl-, O,N-diacetylneuraminic acids and fucose. Such variations in reactivity by neuraminidases towards different neuraminic acids have been recorded in the literature<sup>72</sup>. The oligosaccharide structures attached to these proteins are the code for destruction which is neutralized by the terminal sialic acid residues.

If the situation observed for plasma proteins is subsequently found to apply to other groups of glycoproteins, then each group would be catabolized by a limited number of cell types. This could explain why basically only a few types of oligomeric units are found in glycoproteins<sup>1,2</sup>. Each group would possess a common type of carbohydrate prosthetic group and small variations in the internal sugars could specify the protein for disposal by different cell types. The binding of hydrophobic hormones, for example, cortisol, oestradiol and thyroxine, to specific plasma proteins which is believed to be advantageous on solubility considerations may have a further function in imparting antigenic properties by virtue of the glycoprotein to which the hormone is attached. This can be used to provide organ specificity or to control the rate of removal from the circulation.

The significance of the glycosyl residues is to impart a discrete recognitional role on the protein. This concept of recognition has two facets: the immediate and obvious positive recognition and an equivalently important one in an extension of non-recognition, namely anti-recognition. We envisage this anti-recognition process as not only the hiding of a particular antigenic determinant but also the occlusion of a larger portion of a protein as, for instance, in the prevention of a natural self-aggregation process, for example,  $\kappa$ -casein.

The use of such alterations in the compositions of oligosaccharide units has been demonstrated already in the cellular recognition phenomena<sup>46,73,74</sup>. Robbins<sup>75</sup> has shown that the specificity of interaction of a bacteriophage and a bacterial cell depends on a membranous glycolipid. Genetically engendered alteration of these sugar residues by the phage leads to subsequent non-recognition by the phage and a new recognition by another phage. Similarly, the binding of certain viruses with cells involves the sialic acid residues of surface glycoproteins<sup>73</sup>, the ensuing action of these viruses possibly leading to alterations in the cell coat carbohydrates and the glycosyltransferases<sup>25,76</sup> responsible for their synthesis. The wide ranging effects exhibited by various cell types after neuraminidase treatment<sup>73</sup> endorse the important role that the sialic acids play in the interaction of a cell with its environment. The observations which are often consistent with the sialic acid neutralizing an underlying antigen are consonant with the function proposed for this sugar in the plasma glycoproteins.

Roseman<sup>16</sup> has proposed that intercellular adhesions are a result of a mutual interaction between oligosaccharide units in the membrane of one cell with glycosyltransferases present in the membranes of the adjacent cells. If the oligosaccharide unit is the "substrate" for the transferase, positive binding occurs; if not, the association is abortive. The glycosyltransferases, like the membrane glycoproteins, are incorporated through the Golgi apparatus and are therefore under genetic control. The interaction between cells can also be modified by the provision of the second substrate, the nucleotide sugar, for the enzyme either by *de novo* synthesis or translocation to the active site. It is inherent in this concept that one cell is modifying its neighbour. Experimental evidence in support of this contention has been furnished by work on fibroblasts<sup>25</sup> and platelets<sup>77</sup> in which glycosyltransferases have been located in the plasma membranes of these cells. These glycosyltrans-

ferases could represent the receptor sites for the soluble glycoproteins committed for catabolism.

The inclusion of a limited number of carbohydrate residues can subtly modify the biological function of a particular polypeptide, whether it be an enzyme or a hormone. Carbohydrates are ideal for generating compact units with explicit informational properties since the permutations on linkages are larger than can be achieved by amino-acids and, uniquely in biological polymers, branching is possible. Moreover, the oligosaccharide units are not flexible but exhibit highly specific structures with only limited degrees of freedom<sup>78,79</sup>. A rigid framework is required for these receptor-specific structures in order to maintain the steric interrelationships. This conformational inflexibility within the polypeptide chain of many extracellular proteins is achieved by the incorporation of disulphide bridges.

The carbohydrate need not constitute the entire determinant but may be used to modify the determinant regions of the polypeptide, the final specificity being a result of an interaction of carbohydrate, protein and lipid components. This may be mediated or reinforced by the use of divalent or transition metal cations<sup>80</sup>. In this way precise intracellular as well as extracellular locations may be specified by incorporating particular oligosaccharide units either into the protein or membrane.

We suggest that there is no substance in the belief that carbohydrates are added as passports for export from the cell, but rather that sugars are included in protein structures as a means of coding for the topographical location within an organism. This may either be to effect recognition of a protein for its target cell or, as in the case of the metabolism of plasma proteins, the code may be hidden and not operate until activation by removal of the terminal residues. This would explain why the great majority of extracellular proteins are glycosylated: such proteins by definition have been synthesized in one cell type but fulfil their function at another locus.

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# Cellular Interactions in Mass Cultures of Human Diploid Fibroblasts

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**Fibroblasts in mass cultures generate self-maintaining parallel arrays. In the presence of accumulated collagen, arrays migrate over one another to build metastable orthogonal multilayers. The cell partaking in these interactions is the fibroblast in the bipolar spindle form, which does not exhibit contact inhibition of movement.**

HUMAN foetal lung fibroblasts, maintained in Petri dish cultures without subculturing<sup>1,2</sup>, grow to high densities to form a membranous tissue. During the formation of this membrane from a random seeding, a primitive morphogenesis occurs, the cells assembling into structures of two kinds. There are (1) parallel arrays in which each cell lies parallel to its immediate neighbours, and (2) orthogonal multilayers consisting of a stack of superimposed parallel arrays, with the cells of adjacent layers oriented roughly perpendicularly to each other.

## Morphogenesis in Fibroblast Cultures

Human foetal lung fibroblasts grow beyond confluence to a final density of about  $10^7$  cells/500 mm plate. This cell density is about six to seven times that of a confluent monolayer<sup>3</sup>. We found that once confluence is attained virtually all the cells from further cell division are incorporated within a parallel array; a few are temporarily trapped within the interstices between arrays. The confluent monolayer consists of a patchwork of many independent parallel arrays, and the line of demarcation between adjacent arrays is termed a frontier, across which there is no exchange of cells. Where the cells in adjacent arrays come to within  $20^\circ$  of sharing the same orientation, the frontier between them disappears and the arrays merge into a single larger array.

During growth of the culture after confluence, the parallel arrays of the patchwork overgrow one another. The details of this overgrowth depend on the sizes of the arrays, their shape, and the angle at which cells in adjacent arrays confront one another. This overgrowth gives rise to numerous, usually bilaterally symmetrical, ridge-shaped aggregations termed orthogonal multilayers, consisting of many superimposed monolayers of cells in parallel array with the orthogonal alternation already described (Fig. 1a and b).

During prolonged maintenance of stationary cultures over months, cells of the orthogonal multilayers are gradually dispersed, and the patchwork pattern is simplified by merging in the basal layer of the culture. This results in the formation of very large parallel arrays, which are perfectly stable<sup>4</sup>. Indeed, a whole culture may come to exist as a single parallel array.

## Formation of Orthogonal Multilayers

There are two possible mechanisms by which orthogonal multilayers may be formed: differential growth or cell migration. The migration of cells in parallel array over other cells during the formation of orthogonal multilayers can be demonstrated by time-lapse films. Furthermore, the primary importance of cell migration is confirmed by the following experiment. Orthogonal multilayers do not develop in post-confluent cultures so long as they are maintained beneath a 2% agarose-stiffened medium, which restrains cell motility without interfering with growth. After an agarose overlay has been removed from a stationary culture (in which net growth has ceased), orthogonal multilayers develop within 2 days. It seems then that orthogonal multilayers are formed by cell migration, and differential growth is unimportant.

We now describe the conditions necessary for the generation of orthogonal multilayers. (1) Fibroblasts attain complete surface coverage before overgrowing one another. Orthogonal multilayers are not observed in cultures of normal fibroblasts that cease growth soon after confluence (such as those from adult skin tissue). In these cultures, there is some spillage of cells across frontiers in the confluent patchwork to give occasional sites of ragged overlapping. (2) The large parallel arrays formed in long-maintained cultures after orthogonal multilayers have disappeared are perfectly stable. Although no new orthogonal multilayers developed naturally, they formed around frontiers between surgically-contrived, non-parallel confrontations<sup>1</sup>. (3) The generation of orthogonal multilayers can be prevented by supplementing the medium on confluent cultures with low concentrations of bacterial collagenase. Collagen accumulation (but not growth) is inhibited, and the form, motility and anchorage to plastic of the cells are not visibly affected. In the presence of collagenase, cells (produced after confluence has been attained) are accommodated within the patchwork pattern of numerous arrays and the frontiers become very clearly defined, appearing as ditches between raised banks of cells (Fig. 1c). There is, in this case, no orthogonal overgrowth.

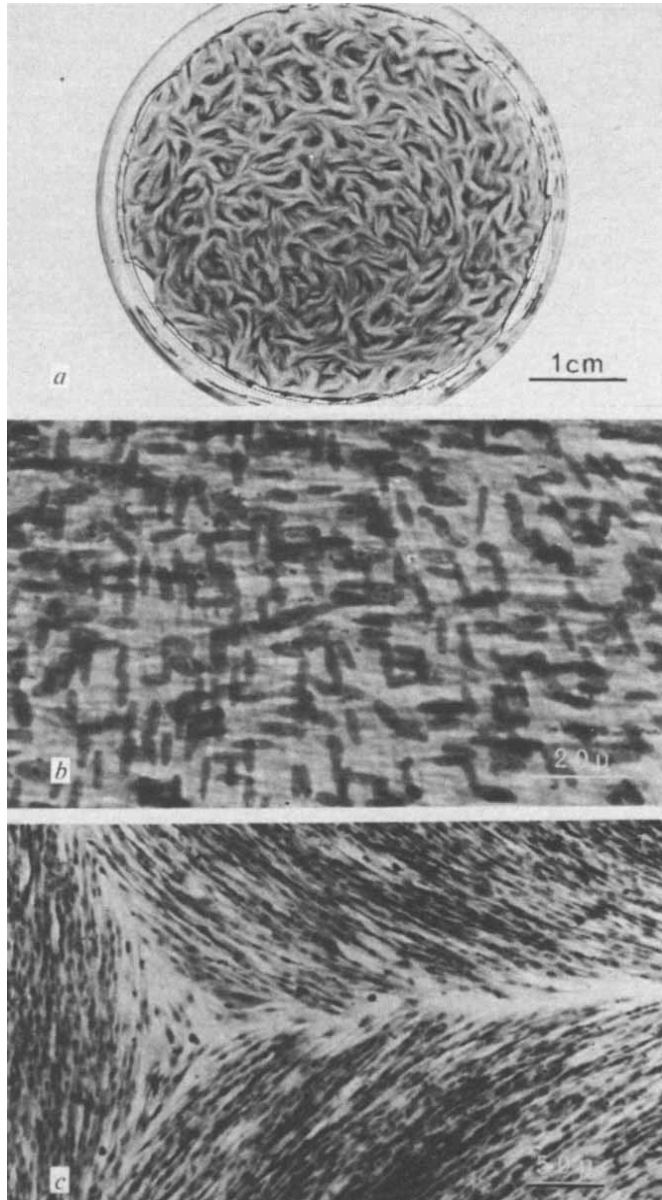
When collagenase is added to stationary cultures containing orthogonal multilayers, the orthogonal patterns are disrupted because all the cells not incorporated within the basal layer are rounded up.

Electron microscopic examination of vertical sections of stationary cultures, grown on conditioned 'Araldite' substrata, shows that the fibrous material, which Goldberg and Green<sup>5</sup> assumed to be collagen fibril precursors, is associated with not more than 30% of the linear cell surface.

## Fibroblast Form

During the growth of sparsely initiated cultures there is a change in the form of the cells as they progress from lone isolation to incorporation within the confluent monolayer. Sparse cells exhibit the "ruffling membrane form" (Fig. 2a).

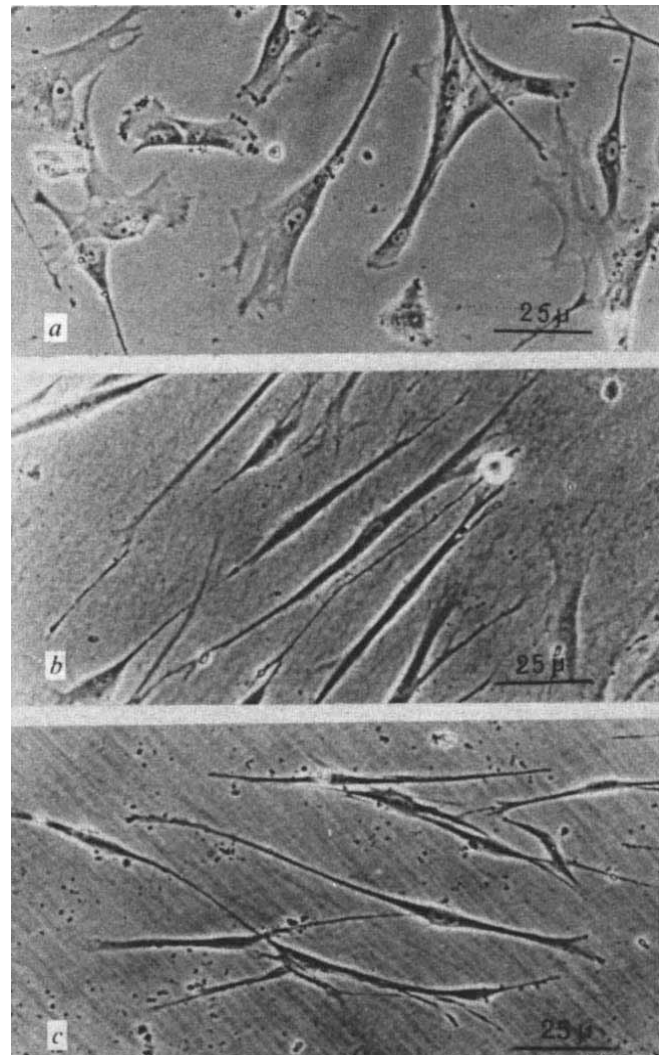
The leading edge of the cell is expanded into a thin, broad fan, the surface of which shows a characteristic activity in phase-contrast time-lapse films. More than one of these transient ruffling membranes may be present; they can apparently be produced from any point on the cell surface. Ruffling membrane forms vary greatly in outline.



**Fig. 1** *a*, Human foetal lung fibroblasts. Three week old, stationary culture grown from a sparse plating, fixed, and stained with haematoxylin. Only cell nuclei are stained and the cells are unevenly distributed; numerous, mainly ridge-shaped prominences (up to ten or twelve cells thick) and thinner intervening regions (down to a monolayer). This uneven distribution results from cell migration during the period of growth after confluence. *b*, A portion of a culture fixed, and stained with haematoxylin. A thin region of the orthogonal multilayering that provided the internal structure of the ridges in *a*. *c*, Petri dish culture of human foetal lung fibroblasts cultured in the presence of collagenase, fixed and stained with haematoxylin. No ridges or orthogonal multilayers. All the cells in this stationary culture are accommodated in the patchwork of parallel arrays established at confluence. The meeting point of three such arrays provides a three-armed discontinuity or frontier. Normally the cells would cross the frontier and overlap, but here they are prevented from so doing by collagen deprivation. The destabilization of the pseudopodia at the frontier results in complete contact inhibition.

As confluence is approached there is a gradual change to the "bipolar spindle form". The cell is extended into a straight spindle; small, unexpanded, spike-like pseudopodia are confined to the two ends of the cell. Virtually all of the cells in confluent and post-confluent fibroblast cultures are bipolar spindles; the ruffling membrane form is not normally represented.

The pseudopodia of the cells confronting one another across a frontier are abnormally expanded to resemble ruffling membranes.



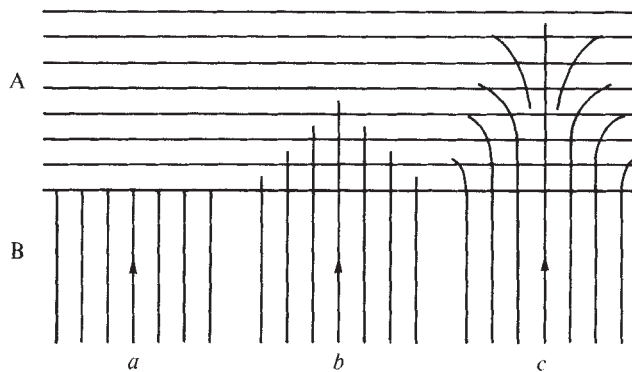
**Fig. 2** Human foetal lung fibroblasts on different substrata, phase contrast microscopy, living cells. *a*, Seeded sparsely onto pristine plastic. Ruffling membrane form. Variable, changing outline; broad, expanded pseudopodia. *b*, Seeded sparsely onto the surface of a hydrated collagen lattice. The majority of cells are in spindle form. *c*, Outgrowth onto exudate coated plastic in a long established culture. Rather regular spindle form. Note especially the small compact pseudopodia. The background lines are machine marks on the underside of the 'Nunc' dish.

After making two series of parallel cuts across a dense cell sheet, the wound edges retract, exposing the surrounding plastic. Cells migrate from the retracted wound edges to recolonize these areas. These cells are moving over plastic coated by the exudates left behind by the previous occupants<sup>6</sup>, and isolated migrants are usually observed in spindle form (Fig. 2c). In contrast, trypsinized cells seeded sparsely onto the same surface first assume the ruffling membrane form.

We reconstitute hydrated collagen substrata containing around 0.1% by weight of collagen as native bundles with



the 640 Å period. Seeded sparsely from a trypsin-prepared cell suspension, most cells extend in the bipolar spindle form (Fig. 2b), following the alignment of the larger collagen bundles in the surface of the substratum.



**Fig. 3** Diagram to illustrate overlapping and divergence in the formation and disappearance of orthogonal multilayers, see text. *a*, Perpendicular confrontation between two parallel arrays. *b*, Array "B" is free to migrate over "A". *c*, Divergence. When as a result of divergence overlapping "B" cells share the same orientation as underlying "A" cells, the "B" cells merge with "A" and are thereby lost from the overlap.

Our purpose is to enquire into the nature of the cellular interactions responsible for the parallel arrays and orthogonal multilayers observed in mass fibroblast cultures.

The cell that partakes in these interactions is the bipolar spindle. The basic difference between this form and the ruffling membrane form is to be found in the stability of their cell surfaces. Time-lapse films show that the spindle form possesses a stable differentiation between pseudopodial and non-pseudopodial surface, absent from the ruffling membrane form. This difference in stability is also reflected in the pseudopodia themselves: small, and uniform in the case of bipolar spindle forms; expanded, active and variable in ruffling membrane forms.

Three especially unnatural conditions accentuate the ruffling membrane form: isolation, attachment to pristine glass and plastic surfaces, and collagen deprivation.

The descriptions of contact inhibition provided by Abercrombie and others derive from observations in ruffling membrane forms.<sup>7-9</sup> The arrest of cell movement at the frontiers in cultures within which overgrowth is inhibited by collagenase appears to represent a phenomenon similar if not identical to the contact inhibition of movement described by these authors. This phenomenon is, however, only observed in our cultures in the unnatural condition of collagen deprivation.

## Parallel Arrays

Our principal concern here is with interactions in confluent cultures<sup>2,4,10</sup>, in which the basic organizational feature is that every cell is incorporated into a parallel array. The arrays in the basal layer of a culture are attached to the plastic. Cells in the supernumerary arrays contributing to orthogonal multilayers are not attached to the plastic; they are rounded up by collagenase; and their extension therefore depends on collagenase-sensitive anchorages.

How are the parallel arrays maintained? If the arrays were static structures, it would be appropriate to seek a mechanism that locked the cells into place within the arrays. The cells, however, are moving, even within dense arrays. Observing the effacement of blemishes and the gradual perfection of initially imperfect arrays formed on partially aligned substrata, it is apparent that cell movement serves to

perfect and reaffirm the parallel configuration. Cell movement is a necessary factor both in the generation of arrays and in safeguarding their proper maintenance in an interfering world.

The maintenance of parallel arrays therefore depends on the directional control of cell movement. Regarding this control we wish to point out the two options principally available.

Contact inhibition operating in this context provides an example of the first option—"return control". A departure from strict parallel is here assumed to create contact-inhibiting encounters as a result of which the situation is returned to normal. As there can be no inhibiting encounters until a departure from normality has occurred, such a control implies that the cells are free to make small excursions from normality. A second implication is that over a certain range, increasing departures from normality evoke a stronger controlling influence.

An example of the second option would be provided by the existence of lateral adhesion with slippage between cells. As lateral contacts are maximized in strict parallel arrays the control here exerts its maximum effect to counteract initial departures from normality. There will exist a range over which the strength of control declines with increasing departures. This is an example of "initial departure control".

These two basically different types of control are complementary and supportive. Initial departure control appears especially appropriate in connexion with the self-maintenance of parallel arrays. Electrostatic forces between cells allow for lateral adhesion with slippage. Experimental demonstration of the importance of this interaction in fibroblast cultures will be provided.

## Orthogonal Multilayers

Three considerations lead to the conclusion that the generation of orthogonal multilayers is appropriately viewed in terms of the interaction between parallel arrays rather than in terms of the interaction of individual cells: (1) orthogonal multilayers consist of parallel arrays; (2) they are constructed out of parallel arrays; and (3) no interaction leading to the development of orthogonal multilayers is possible given a single parallel array. Not until a second array is generated within a large array as a result of surgery is an interaction possible.

Interactions between parallel arrays are governed by a simple rule. This rule states that where the axes of cellular elongation in two adjacent arrays make an angle of more than about 20° the arrays remain separate (there is no exchange of cells between arrays), where the angle is less than about 20° the arrays merge into one. This rule governs the interaction of arrays in both the horizontal and vertical planes. In the horizontal plane it governs the formation of progressively larger arrays formed as a result of merging.

Interactions in the vertical plane are responsible for the formation and disappearance of orthogonal multilayers. Consider the situation in Fig. 3a, where one array confronts another perpendicularly. Cells in array "B" are free to migrate over "A" to form an initially perpendicular overlap (Fig. 3b). Cells in A encountering the overlap in the course of their to and fro movements are free to overlap and overlap, more B array cells can overlap them, and so on. Population pressure within the underlying arrays alone limits the number of overlaps.

Consider now the advancing front of the first overlapping B array cells. These cells are no longer incorporated within the confluent monolayer. They show the same behaviour as an outgrowth of fibroblasts advancing over an uncolonized substratum: the cells in the centre of the front tend to move straight on, the cells in the lateral regions of the front diverge in their movements leftwards and rightwards to form a fan shaped outgrowth (Fig. 3c). When this divergence brings B array cells close to sharing the same orientation as A array

cells, the B array cells merge with A and are thereby lost from the overlap.

This illustrates the essential processes involved in the formation and disappearance of orthogonal multilayers: free migration of arrays over other arrays, divergence, and a rule governing merging.

This view of phenomena accommodates the following facts and considerations. (1) Observed multilayers are predominantly orthogonal or nearly so. (The orthogonal overlap is the most stable. Cells are more readily lost from non-orthogonal overlaps by divergence and merging.) (2) Multilayers eventually disappear (due to divergence and merging). (3) The collagen present is not considered to play any role in directing cells into the orthogonal pattern.

The last point requires further comment. Advocates of a directing role for the collagen have to reconcile (a) that fibroblasts align parallel to collagen bundles in their sub-stream, with (b) that in tendon and muscle the parallel arrayed connective tissue elements produce a matrix in which collagen bundles share the same orientation as the cells (as in the regeneration of tendons<sup>11,12</sup>). Furthermore, the collagen observed between the cell layers in sections of fibroblast cultures viewed in the electron microscope is not aggregated into bundles with a lateral periodicity. The collagen appears to be in the form of fine fibres. It is possible that these fine fibres are too thin to have an aligning influence on cells.

As for the permissive function of collagen in the generation of orthogonal multilayers, the fact that there is no

visible fibrillar material associated with the larger proportion of the cell surfaces speaks against collagen providing a layer insulating superimposed monolayers from mutual contact. It is suggested that the collagen contributes to the stabilization of the bipolar spindle form not subject to contact inhibition, and also provides for the anchorage of cells in the supernumerary layers.

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# Occurrence and Significance of Formaldehyde in the Allende Carbonaceous Chondrite

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**Meteorites may disperse formaldehyde to planets where, in the proper conditions, the compound could then serve as a precursor for carbohydrates.**

THE fall of the Allende meteorite in northern Mexico on February 8, 1969, was observed by thousands of people over a large area, and many individual stones, presumably fragments of a single, large meteoroid, were recovered within a few days. At least 2 tons of specimens were collected within about 5 months of the fall, with individual specimens weighing as

much as 110 kg<sup>1</sup>. The meteorite has been classified as a Type III carbonaceous chondrite<sup>2</sup>.

A small group of five specimens have unusual lustrous fusion crusts—a particularly dramatic example is the “antler” shown in Fig. 1 (ref. 1). These fusion crusts enclosed or were filled with what has been described as a “colourless hydrocarbon material”. It seemed to us possible that any organic material near the surface of the meteorite might have been pyrolysed to produce a liquid or vapour which was driven towards its interior as it entered the Earth’s atmosphere. On cooling, this material would have been drawn into the previously hot and evacuated hollow “antlers”, or penetrated and filled the blisters of lustrous fusion crust. It was the objective of this study to determine the character of this material.



Before analysing the organic substance or substances associated with the lustrous fusion crust, however, we wanted to establish the forms of carbon in the meteorite in order to gain a better insight into the nature of any material that might have served as a source. The results of this examination warrant description now, although analysis of the liquid organic material associated with the fusion crust has not yet been carried out.

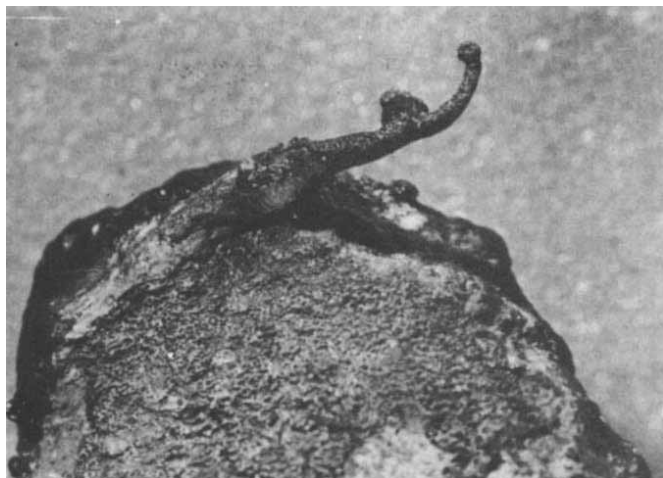


Fig. 1 Fusion crust features illustrating "antlers". ( $\times 4$ , from ref. 1.)

Initial studies were conducted on two fragments of the meteorite (NMNH 3529, 2,570 g; NMNH 3559, 596 g). Pieces of specimen NMNH 3529 were chosen because they have been and are being subjected to detailed chemical and mineralogical studies in other laboratories. Each specimen was carefully cut with a diamond saw to remove all outer surfaces. Distilled water was used to cool the blade during the sawing operation, the saw itself having previously been scrubbed with water and then washed with carbon tetrachloride. The sawing operation removed all fusion crust and any surfaces that may have been contaminated when the stones landed. The trimmed specimens were individually crushed to particles smaller than approximately 5 mm using a pre-washed steel mortar and pestle. Every effort was made throughout these operations to minimize contamination of the specimens.

## Demineralization

The Allende meteorite contains approximately 0.3% carbon<sup>1</sup>. Our initial objective was to isolate any carbonaceous substances represented by this analysis for identification. We used hydrofluoric and hydrochloric acids for decomposition of the silicate minerals. Pure hydrofluoric acid was prepared from hydrogen fluoride by passing the gas through a 'Teflon' cylinder packed with 'Teflon' shavings. Both shavings and cylinder were washed with redistilled benzene before use. To produce the acid, the exit gas was directed, by a stainless steel tube fitted with a platinum tip, into a 500 ml. platinum dish approximately half full of distilled water. Titration indicated the concentration of acid, which was prepared as required, to range from approximately 48 to 60% by weight. Hydrochloric acid (ACS reagent) was purified by distillation in a glass still and recovery of the constant boiling mixture.

The two crushed samples (NMNH 3529, 100 g; NMNH 3559, 94 g) were each divided into approximate quarters, and each quarter was placed in a 200 ml. platinum dish with approximately 100 ml. of hydrofluoric acid. These mixtures were heated on a steam bath with stirring and frequent addition of fresh acid

for several weeks. Precautions were taken to prevent introduction of dust or extraneous matter. At the end of this period, all the residues were combined and the hydrofluoric acid was replaced by incremental additions of hydrochloric acid, each addition but the last being followed by evaporation. This acid was decanted and replaced with fresh hydrochloric acid, the procedure being repeated until the decantate was no longer coloured by dissolved iron. The final product was washed with distilled water until the washings reached a pH of 6.7, and the residue was dried under vacuum at 65° C to yield 0.7 g of product which, on analysis, still had an unacceptably high ash content.

This product was re-treated for several weeks with a mixture consisting of about nine parts of hydrofluoric acid per part of hydrochloric acid. Following the procedure outlined above, 0.54 g of material was recovered and 100 mg of this product was ashed in an oxygen plasma in a low-temperature asher. The final ash content was 32%, a value that could not be reduced further. Duplicate analyses of 0.5 mg samples proved them to contain 65.5 and 65.6% carbon, no hydrogen and no nitrogen. These data pointed to an approximately 60% recovery of the carbon in the original samples. The ash and carbon values account for 97.5% of the sample; the remaining 2.5% can be accounted for by small losses that were observed when the sample was mixed during the ashing procedure. X-ray diffraction analysis of this product provided no indication of graphitic structure, showing the material to be amorphous carbon. The X-ray diffractogram of the carbon isolate, as well as that taken on the ash recovered from the low-temperature ashing procedure, showed reflexions indicative of chromite. Semiquantitative spectrographic data for the low-temperature ash showed major concentrations of chromium and iron, confirming the chromite; minor residual quantities of magnesium, silicon and aluminium were also present.

To confirm these results, 100 g of the crushed sample (NMNH 3529) was treated as before but with a 1:1 HF-HCl mixture for several weeks. The product (0.203 g) now had a higher carbon content, 79.8%, but, as before, there was no evidence of hydrogen or nitrogen. X-ray diffraction analysis again showed the product to consist of amorphous carbon and to contain chromite. Duplicate carbon isotope analyses gave values of  $-16.07$  and  $-16.25$ ‰ for  $\delta^{13}\text{C}$  (PDB) (J. Lawless, private communication). These values can be compared with those for the acid-insoluble carbon from the Mokoia and Lancé Type III carbonaceous chondrites where values of  $\delta^{13}\text{C}$  (PDB) are  $-18.0$  and  $-23.4$ ‰, respectively<sup>3</sup>.

The absence of hydrogen and nitrogen in the "organic" isolate points to the absence of macromolecular compounds which, on decomposition, might have provided the organic filling of the fusion crust. If our theory regarding the origin of this filling were correct, some organic matter must have been present in the meteorite. Thus some low molecular weight organic material might well have been present but may have been lost during the isolation procedure, perhaps by solution in the aqueous acids. This point was checked by pyrolysis.

The ground meteorite (10 g) was placed in a 20 mm horizontal 'Pyrex' tube approximately 10 cm long sealed to a vertical 3 mm tube approximately 10 cm long. The glass unit was evacuated with a mechanical vacuum pump and sealed under vacuum. With the 3 mm tube immersed in dry ice to serve as a condenser, that part of the tube containing the meteorite was heated to red heat in about 5 min. After cooling, the condenser was removed from the dry ice and found to contain a small amount of a yellowish liquid that fluoresced light blue under an ultraviolet lamp. To obtain sufficient material for analysis, the procedure was repeated with 37 g of ground meteorite. A small amount of the distillate from this experiment was mixed with spectroscopic potassium bromide and an infrared absorption spectrum (Fig. 2A) was obtained using a 1.5 mm KBr disk and a Perkin-Elmer model 21 spectrophotometer equipped with a  $\times 6$  double beam condenser. Comparison of

the spectrum of the unknown with published spectra showed the unknown to have most of the features of an aqueous solution of formaldehyde<sup>4</sup>. This was confirmed by obtaining a spectrum of an aqueous solution of formaldehyde (ACS reagent) in our laboratory using Irtran 2 cells (Fig. 2B). In comparing the spectra of Fig. 2, it must be kept in mind that the aqueous solution (B) contains 10 to 15% methanol as a preservative, and that the methanol undoubtedly reacts with the formaldehyde to yield a complex mixture of products that affect the appearance of the absorption curve.

Formaldehyde tends to polymerize to *para*-formaldehyde, which for the most part is a complex mixture of compounds with the empirical formula:



The value of  $n$  varies depending on temperature, concentration (if the formaldehyde is in aqueous solution), and other factors<sup>5</sup>. Polymerization leads to the disappearance of the carbonyl group, which would normally be expected to show up at about  $1,750\text{ cm}^{-1}$ . The ability of formaldehyde to form sugars is also well known<sup>5</sup>. Interrelationships between formaldehyde and *para*-formaldehyde are further complicated by the fact that these two substances yield very different infrared spectra (see Figs. 2B and 3D).

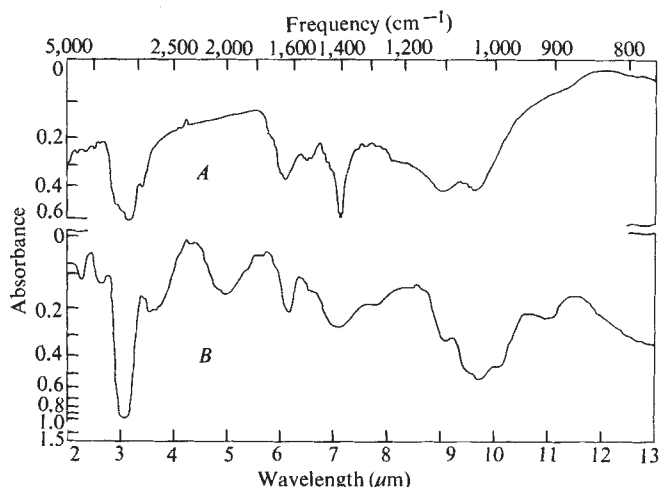


Fig. 2 Infrared spectra: A, distillate from meteorite; B, aqueous solution of formaldehyde (ACS reagent: 36–38% formaldehyde containing 10 to 15% methanol as preservative). Perkin-Elmer model 21 spectrophotometer, sodium chloride optics. Curve A was obtained using a  $\times 6$  beam condenser.

Is the formaldehyde (*para*-formaldehyde) obtained by heating the meteorite a pyrolytic product or does it occur as such in the sample? To resolve this question, crushed meteorite (NMNH 3529, 50 g) was refluxed with 100 ml. of redistilled water for 2 weeks under filtered, high purity nitrogen, and the opalescent solution was decanted and centrifuged. The clear, supernatant liquid was evaporated slowly at approximately  $50^\circ\text{C}$  under a stream of helium to yield a pale yellow, solid residue. This material was analysed in triplicate and found to contain 3.82, 3.77, 3.68% carbon, and 0.61, 0.66, 0.63% hydrogen. The hydrogen-carbon ratio based on this analysis is  $-\text{CH}_2-$ . It was not possible to obtain a direct oxygen determination for this material but, if the product were assumed to be formaldehyde ( $\text{CH}_2\text{O}$ ), then the solid isolate would consist of 9.4% formaldehyde (in the form of *para*-formaldehyde) and 90.6% inorganic matter. An infrared spectrum of the total extract (both organic and inorganic) is shown in Fig. 3C. Curve D of Fig. 3 is for commercially available *para*-formaldehyde and

illustrates the difficulties inherent in attempting to make a positive identification of formaldehyde by means of its infrared absorption spectrum. Again, however, as for curves A and B of Fig. 2, emphasis is placed on the absence of a carbonyl absorption peak at about  $1,750\text{ cm}^{-1}$  in both the spectrum of the extract and that for a solution of formaldehyde.

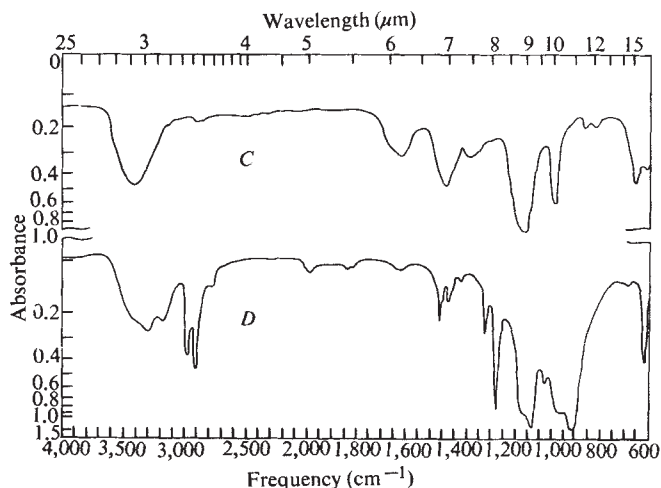


Fig. 3 Infrared spectra: C, total residue from aqueous extract from meteorite. This includes soluble inorganic material. D, *para*-Formaldehyde (Fisher Scientific Co.). Perkin-Elmer model 621 spectrophotometer, KBr disks. Curve C was obtained using a  $\times 6$  beam condenser.

To confirm the presence of formaldehyde (as *para*-formaldehyde), approximately one-third of the extract was treated with several millilitres of methanol to precipitate the inorganic substances. This methanol extraction was repeated several times, and the total extract was then carefully reduced in volume at approximately  $50^\circ\text{C}$  under a stream of helium, the methanol being removed by alternate evaporations and additions of water; the solution was never permitted to go to dryness. The aqueous extract was now diluted to 50 ml. with water in a volumetric flask. When this solution was allowed to react with *p,p'*-phenylenediamine in the presence of hydrogen peroxide<sup>6</sup>, a brownish colour developed for final confirmation of the presence of formaldehyde. Quantitatively, this test indicated the presence of approximately 3 p.p.m. of formaldehyde in the meteorite.

*para*-Formaldehyde does not respond to this colour test, which has been reported to be extremely selective for formaldehyde, and it was in part to depolymerize the *para*-formaldehyde to formaldehyde that we heated the extract when the methanol was replaced by water. Also, it was noted in preliminary tests that the inorganic material present in the aqueous extract prevented formation of the colour reaction. It was also to remove the inorganic material that the extract was treated with methanol. Although the colour reaction is able to detect as little as  $0.05\text{ }\mu\text{g}$  of  $\text{HCHO}/\text{ml}$ ., our solution contained  $0.5\text{ }\mu\text{g}$   $\text{HCHO}/\text{ml}$ .

Parallel blanks were run throughout these studies. It is interesting to note that rapid disaggregation of the meteorite into extremely small, separate mineral particles occurs when the sample is refluxed with water. This contrasts with reports that significant disaggregation is very difficult to achieve by mechanical means.

## Significance of Formaldehyde

It is now clear that the organic material associated with the fusion crusts was probably derived from the formaldehyde



(which is assumed to occur as *para*-formaldehyde in the meteorite) together with small quantities of hydrocarbons that have been shown to be present in the meteorite.

The origin of the formaldehyde is worthy of speculation. It could represent part of the initial agglomeration of the meteorite and might therefore be of a juvenile character. Alternatively, formaldehyde is known to exist in interstellar space<sup>7</sup> and it is possible that the meteorite absorbed the compound during its travels. A third possibility is that the formaldehyde was synthesized photochemically or radiochemically on the surface of the meteorite from an appropriate mixture of adsorbed gases. And formaldehyde, as a product of our terrestrial environment, may have been absorbed by the meteorite during its fall through the Earth's atmosphere. This last source seems unlikely, however, especially as the meteorite was hot enough to have had a molten silicate skin as it passed through the Earth's atmosphere.

Still another possibility must also be considered; formaldehyde might have been produced and absorbed if the meteorite pyrolysed the material on which it landed. All reliable evidence points toward the fact that when specimens of the meteorite landed on dry grass they had already cooled to the point where no charring occurred. The meteorite contains no absorbed water<sup>1</sup>, water being the most prevalent substance available for absorption in these conditions, so that it seems highly unlikely that formaldehyde would have been derived from this source. The absence of water similarly militates against the absorption of formaldehyde in the upper atmosphere where water still prevails, also suggesting that Levy and his co-workers<sup>8</sup> were correct when they concluded that the hydrocarbons they volatilized from the meteorite were indigenous and not contaminants.

The occurrence of formaldehyde indicates a relatively low-temperature accretion and subsequent history. This evidence supports a recent conclusion that the chondrules in this meteorite underwent cold accretion<sup>9</sup>.

## Formaldehyde and the Origin of Life

Amino-acids, some sugars and other complex compounds have been synthesized by the action of electrical discharge or radiation on gases presumed to have been present in the Earth's primitive atmosphere<sup>10</sup>. It seems that compounds essential to the formation of life could therefore have been made available for the further synthesis of proteins, carbohydrates, and lipids early in the Earth's history, as suggested by Oparin<sup>11</sup> and others. The recent discovery of amino-acids in the Murchison meteorite<sup>12</sup>, and the identification of formaldehyde reported here, give us cause to reconsider some of the broader theories regarding the origin of life.

It is impossible to do more than make an intelligent guess, based on the assumptions in Table 1, regarding the amounts of formaldehyde and amino-acids that might reach Earth daily by meteorite.

Using the values of Table 1, it seems that approximately  $0.5 \times 10^{14}$  g of formaldehyde and  $3 \times 10^{14}$  g of amino-acids could have been deposited on Earth by meteoritic influx during the time from the origin of the Earth to the time when life is thought to have begun. Even if these values are too high by several orders of magnitude, the quantities of these substances are still huge. It must also be kept in mind that during this time there were no organisms present able to destroy the compounds so deposited.

Shapley<sup>14</sup> has estimated the number of planets on which life might evolve as  $10^{11}$  to  $10^{14}$ . Perhaps these recent findings have a special significance regarding theories of the origin of life.

Arrhenius<sup>15</sup> suggested that life could be transmitted by means of spores carried by meteorites (panspermia). Although neither living organisms nor spores seem to be meteoritic constituents, our work shows that precursors for life can be transported through space by certain meteorites. Together with the work of Kvenvolden *et al.*<sup>12</sup> this must portend a

rebirth of Arrhenius's panspermia theory in a revised and currently acceptable form. We may now conclude that certain compounds that exist in space or may be formed on meteoritic surfaces or within meteorites can be distributed by those meteorites and, on landing on a friendly body, may well serve as the precursors of life on that body. Proteins and related substances are to be expected in the case of amino-acids; in the case of formaldehyde, carbohydrates are the anticipated products<sup>16</sup>.

**Table 1** Assumptions Relevant to Amounts of Formaldehyde and Amino-acids reaching Earth Daily by Meteorite

|                                                                                                                      |                   |
|----------------------------------------------------------------------------------------------------------------------|-------------------|
| Daily influx of meteorites on Earth <sup>13</sup>                                                                    | ~ 100 metric tons |
| Daily fall of carbonaceous chondrites (50% of total)                                                                 | 50 metric tons    |
| Content of formaldehyde in carbonaceous chondrites (assuming all such chondrites have the same content)              | 3 p.p.m.          |
| Content of amino-acids in carbonaceous chondrites <sup>12</sup> (assuming all such chondrites have the same content) | 15 p.p.m.         |
| Time between origin of the Earth and origin of life on Earth ( $4.5 \times 10^9$ yr to $3.5 \times 10^9$ yr)         | $10^9$ yr         |

It is not unreasonable to expect that other compounds that have been identified in outer space may also be associated with meteorites. Thus, interactions among the approximately twenty compounds that have been reported may lead to an extensive series of additional life-related compounds in meteorites.

On the other hand, formaldehyde may be unique in its retention in the Allende meteorite. Its peculiar reactions<sup>5</sup>, leading to chains, rings or polyhydroxy compounds similar to sugars, may account for its retention where other compounds now known to occur in space have not been identified in meteorites.

The origin of the amorphous carbon in the Allende meteorite is unexplained; it may represent primordial carbon or a reduced product. Studies of the forms of carbon in this meteorite, as well as of the liquid organic matter associated with the fusion crust, are continuing.

We thank Mrs Janet Fletcher for spectroscopic analyses and Dr James Lawless for carbon isotope studies.

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# LETTERS TO NATURE

## PHYSICAL SCIENCES

### High Resolution Observations of the Submillimetre Stratospheric Emission Spectrum

THIS letter reports the preliminary results of some recent measurements of the thermal emission spectrum of the stratosphere in the submillimetre region. This follows analysis of previous measurements from aircraft<sup>1-3</sup> using Fourier spectroscopic techniques which showed how, using a Michelson interferometer with a Golay cell detector, spectra may be obtained to a maximum resolution of about  $0.2\text{ cm}^{-1}$ . From such results, the mixing ratios of  $\text{H}_2\text{O}$  and  $\text{O}_3$  may be determined relative to  $\text{O}_2$ . Several emission lines due to  $\text{H}_2\text{O}$ ,  $\text{O}_2$  and  $\text{O}_3$  were also observed for the first time in the atmosphere, which raised the possibility that other much weaker lines due to minor atmospheric gases might be observed.

We have now achieved a large improvement in spectral resolution by using a more sensitive cooled detector. Our technique<sup>1,2</sup> uses a Michelson interferometer with phase-modulation<sup>4</sup> for chopping. The instrument is mounted on board the Comet 2E experimental aircraft of Radio Flight, RAE, Farnborough, and the sky is viewed by means of a small Cassegrain telescope, through a polypropylene window. In this work, the viewing angle was  $15^\circ$  above the horizontal. The most important modification in the present experiment was the use of a helium-cooled Rollin InSb electron-bolometer<sup>5,6</sup>, in an all-metal cryostat (Infra-Red Laboratories, Tucson, Arizona), in place of the Golay cell used previously. The detector was optically matched to the interferometer using an  $f/1$  TPX (ref. 7) lens at the interferometer output aperture and a copper light cone (10 mm aperture, 50 mm length, semi-angle  $3^\circ$ ) in the detector. A preamplifier was screened in a box on the side of the cryostat<sup>6</sup> to reduce electrical pick-up. No interference from the aircraft electrical systems was observed, or from vibration (the apparatus was mounted on anti-vibration mounts<sup>3</sup>). For further information on the experimental system we refer to the literature<sup>1,3,4,6</sup>.

Interferograms were recorded to a maximum of 80 mm optical path difference. Interferogram samples were taken at rates of up to  $10\text{ s}^{-1}$ , and recorded to 1 part in  $10^4$  discrimination on paper tape. Typically a smoothing time constant of 100 ms or 300 ms was used. The flights were on November 11, 12 and 16, 1971, and a complete report of the 28 spectra obtained, including a measurement of the  $\text{H}_2\text{O}$  mixing ratio<sup>2</sup>, will be presented later: here we present examples of the high resolution data obtained on the first and second days to indicate the quality of the results and to illustrate the observation of many weak atmospheric emission lines never before detected.

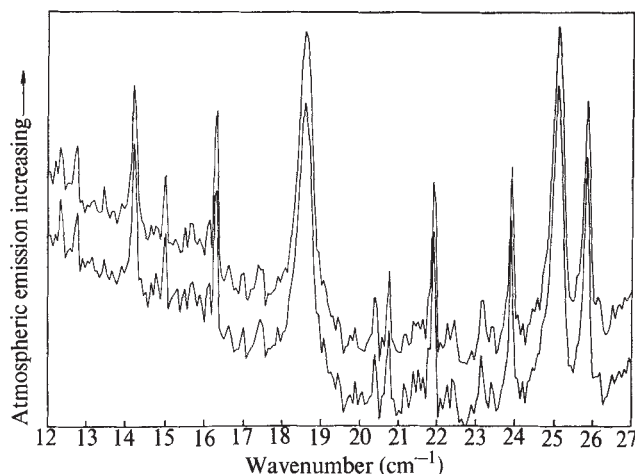
Fig. 1 shows two spectra obtained by a sine Fourier transform<sup>4</sup> of the interferogram data recorded on November 11 at an altitude of 12 km. Only part of the complete spectrum is shown, and the unapodized spectral resolution is  $0.0625\text{ cm}^{-1}$ . As discussed previously<sup>8</sup> we have not found it desirable or necessary to apodize the spectra. These measurements were obtained with a  $50\text{ }\mu\text{m}$  thick beam-splitter, and, as the response of the detector falls off at wavenumbers higher than about  $25\text{ cm}^{-1}$ , the peak instrumental transmission occurs at about

$22\text{ cm}^{-1}$ . The reproducibility between the two runs is very good and the coincidence of many weak lines is apparent, in addition to the known stronger lines<sup>1</sup>. As expected from the quality of the interferograms, the noise level is very low.

The zero of atmospheric emission (that is, the windows between the emission lines) is not flat but is depressed because the metal cryostat itself acts as a radiation source so that radiation from around the window of the cryostat enters the interferometer. Ideally half of the radiation from any source is reflected by a Michelson interferometer back towards that source<sup>9</sup> and is  $\pi$  radians out of phase with the "incoming" sky radiation, thus contributing a "negative" term to the detected power at a given wavenumber. This effect does not, however, confuse line assignments in any way.

By operating the apparatus in the laboratory with a black-body source we obtained "background" spectra, again to an unapodized resolution of  $0.0625\text{ cm}^{-1}$ , which showed clearly that none of the new weak lines are artefacts: some interference fringes do occur, due probably to standing waves in the detector window, but these are very broad (about  $1\text{ cm}^{-1}$ ) and do not lead to any confusion in our assignments. From the laboratory black-body calibrations at various temperatures, it is possible to set fairly close photometric limits to the radiation in our frequency range entering the instrument in flight from sources outside the stratosphere, and a report giving details of new upper limits to the isotropic submillimetre background is being prepared.

Figs. 2a, b and c show that almost all the weak features in the spectrum may be assigned to  $\text{H}_2\text{O}$ ,  $\text{O}_2$ ,  $\text{O}_3$  and, very probably,  $\text{HNO}_3$ . The solid curve is the average of the two runs shown in part in Fig. 1, at an unapodized resolution of  $0.0625\text{ cm}^{-1}$ , in the ranges  $10\text{--}18\text{ cm}^{-1}$ ,  $18\text{--}27\text{ cm}^{-1}$  and  $27\text{--}36\text{ cm}^{-1}$  respectively. Fig. 2a also shows the average of two runs from the second day's flight (November 12—broken curve) at an unapodized resolution of  $0.0667\text{ cm}^{-1}$ . On this day a thicker ( $100\text{ }\mu\text{m}$ ) beam-splitter and additional filtering (3 mm black polyethylene) were used to limit the spectrum to below



**Fig. 1** A comparison of two single runs obtained on November 11, 1971, at an altitude of 12 km. The spectral resolution (unapodized) is  $0.0625\text{ cm}^{-1}$ . Almost all the weak lines may be assigned to  $\text{O}_3$  and  $\text{HNO}_3$ . The reproducibility between the two spectra is excellent.



20  $\text{cm}^{-1}$ : this average is, therefore, more sensitive in the 10–15  $\text{cm}^{-1}$  region and has been included for comparison with the solid curve. The agreement between these independent observations is very good.

The lines of  $\text{H}_2\text{O}$ ,  $\text{O}_2$  and  $\text{O}_3$  which have been previously identified in the stratospheric spectrum<sup>1,3,10</sup> are marked as such above each line. The bars at the bottom represent the theoretical spectrum of  $\text{O}_3$  due to Gora<sup>11</sup>, and are proportional to the peak absorption coefficients quoted by him. Most of the new weak lines which we have observed may be assigned quite definitely to  $\text{O}_3$ : these are marked below the curve by "3". Apart from the well known Q-branches, none of these weaker lines of  $\text{O}_3$  have been observed before, so these results are the first detailed experimental verification of Gora's calculations in the submillimetre region (although note the recent microwave work below 10  $\text{cm}^{-1}$  in ref. 12). There is good agreement in both relative intensities and in line positions between experiment and theory for the lines predicted by Gora, further illustrating the high signal-to-noise ratio and spectral resolution achieved in these results.

Those features which may be assigned to very weak lines of  $\text{H}_2\text{O}$  and its isotopes have also been marked below the curve by "H" (refs. 13–15). All features which then remained unassigned have been cross-hatched: in order to assign these we now consider other gases.

There is great interest in the possible existence in the stratosphere of gases such as  $\text{NO}_2$ ,  $\text{HNO}_3$ ,  $\text{SO}_2$ , and  $\text{N}_2\text{O}$ , particularly from the point of view of stratospheric pollution<sup>16</sup>. Previous calculations (J. E. H., unpublished) of the expected intensities of emission lines due to several minor atmospheric gases showed that at the relatively high elevation angle (15°) used here, only  $\text{HNO}_3$  lines should be observable (based on the concentration figures obtained by Murcray *et al.*<sup>17,18</sup>). We have, therefore, used published laboratory measurements and calculation of the spectrum of  $\text{HNO}_3$  (ref. 19) in our analysis.

Those lines of  $\text{HNO}_3$  which fall in regions fairly free of other lines and which are therefore most likely to be observed have been marked by arrows and labelled "N". Fig. 2 shows that many of the arrowed and cross-hatched features do coincide. Moreover, in no case does a feature not appear where an  $\text{HNO}_3$  line is expected to be visible. In Fig. 2a the two days' observations agree well in nearly all cases. Thus, although not conclusive, the evidence strongly suggests that we are observing emission from  $\text{HNO}_3$ . Calculations of the approximate concentration of  $\text{HNO}_3$  required to explain the magnitude of the observed features, based on laboratory data<sup>19</sup>, indicate a mixing ratio of between 5 and 15 p.p.b. in excellent agreement with the 10 p.p.b. or so obtained by Murcray<sup>17,18</sup>.

We have examined the spectra for evidence of other gases such as  $\text{NO}_2$  and  $\text{SO}_2$  which ought to have more intense emission lines than, say,  $\text{N}_2\text{O}$  or  $\text{NO}$  (which are far too weak to be observed in the present arrangement), but no evidence for these species was found. (The extensive unpublished experimental data of N. W. B. Stone and H. A. Gebbie have been invaluable in these assignments<sup>20</sup>.) For further detailed studies of these minor constituents it will be necessary to increase the effective atmospheric emission path by reducing the elevation angle of observation. Reducing the elevation angle, for example, from 15° to 1° yields a seven-fold increase in path length.

One gas which we cannot at the moment comment on, and which is certainly worthy of investigation, is  $\text{H}_2\text{SO}_4$ . This has not yet been observed in the stratosphere, but may be expected to be present. No submillimetre spectrum for this molecule has, to our knowledge, yet been recorded, and an experimental (and theoretical) study of this species is required before a complete analysis of the stratospheric spectrum may be made.

To confirm the assignments reported here we shall continue the analysis of these spectra and of spectra obtained at different altitudes. Just as we have been able to make quantitative measurement of the concentration of  $\text{H}_2\text{O}$  from our previous

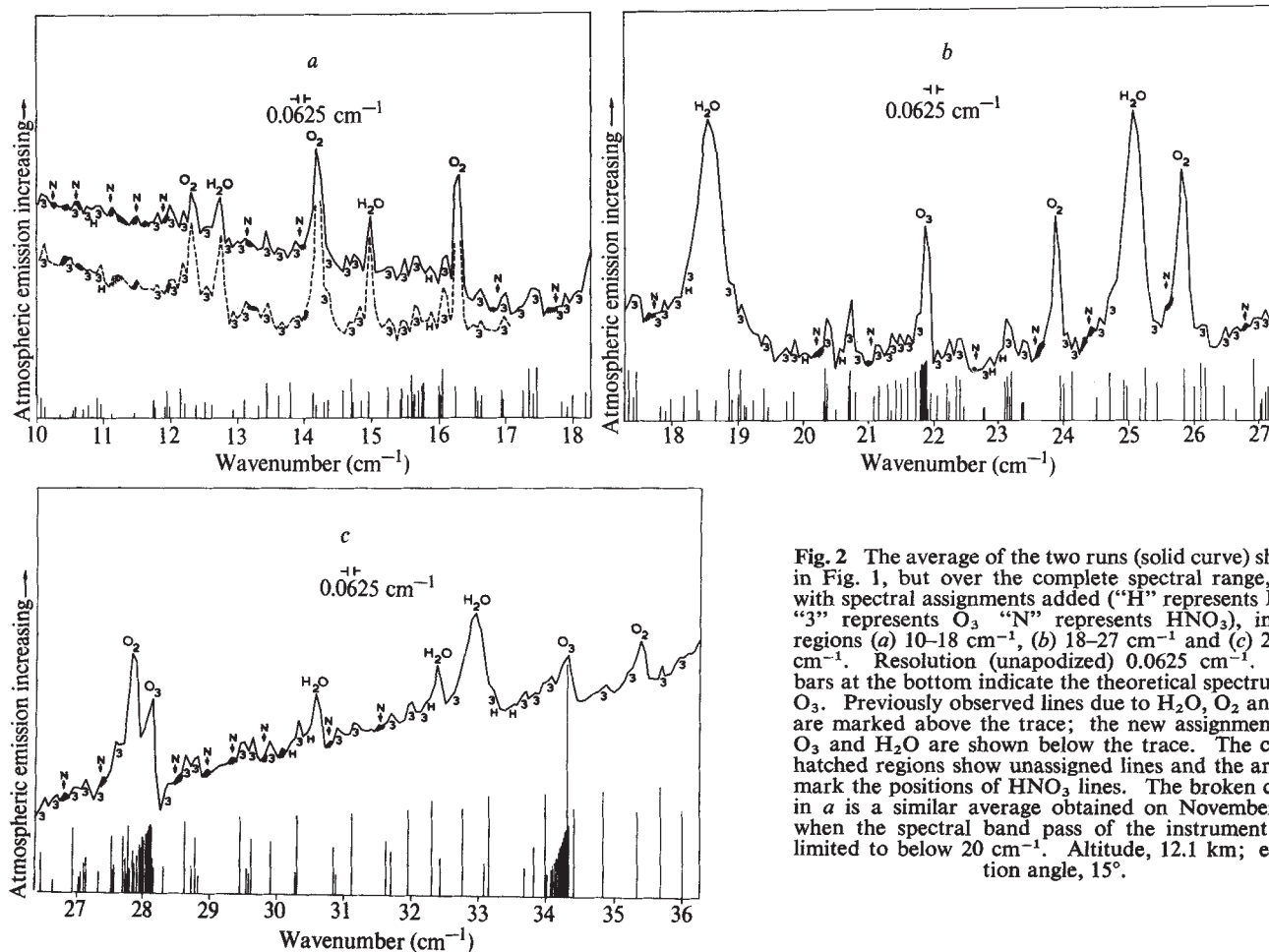


Fig. 2 The average of the two runs (solid curve) shown in Fig. 1, but over the complete spectral range, and with spectral assignments added ("H" represents  $\text{H}_2\text{O}$ , "3" represents  $\text{O}_3$ , "N" represents  $\text{HNO}_3$ ), in the regions (a) 10–18  $\text{cm}^{-1}$ , (b) 18–27  $\text{cm}^{-1}$  and (c) 27–36  $\text{cm}^{-1}$ . Resolution (unapodized) 0.0625  $\text{cm}^{-1}$ . The bars at the bottom indicate the theoretical spectrum of  $\text{O}_3$ . Previously observed lines due to  $\text{H}_2\text{O}$ ,  $\text{O}_2$  and  $\text{O}_3$  are marked above the trace; the new assignments to  $\text{O}_3$  and  $\text{H}_2\text{O}$  are shown below the trace. The cross-hatched regions show unassigned lines and the arrows mark the positions of  $\text{HNO}_3$  lines. The broken curve in a is a similar average obtained on November 12, when the spectral band pass of the instrument was limited to below 20  $\text{cm}^{-1}$ . Altitude, 12.1 km; elevation angle, 15°.

measurements<sup>2,20</sup>, we are now able to do this for much rarer gases, as we have illustrated for  $\text{HNO}_3$ . By choosing the correct observing conditions measurement of minor (and in some cases, pollutant) gases in the stratosphere evidently becomes possible using these techniques.

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## Seismic Refraction Investigation of Deep Sedimentary Basin in the Continental Shelf west of Shetlands

GEOPHYSICAL surveys of the Shetland-Hebridean continental shelf have located deep sedimentary basins<sup>1,2</sup>. During cruises of MV Moray Firth and MV Arran Firth in 1969, and RRS John Murray in 1970, three unreversed seismic refraction profiles were shot over and adjacent to one of these basins, the positions of the lines being fixed relative to the gravity field (Fig. 1).

Gravity, magnetic and sparker data show that crystalline basement rocks crop out at the seabed causing gravity "high" *A* and that a mean basement/sediment density contrast of at least 0.3–0.4 g/cm<sup>3</sup> is required to satisfy the steep gradient between "high" *A* and "low" *E*. Bott and Watts interpret this as a faulted contact between Mesozoic and Lewisian rocks.

The seismic refraction data from line *L* (located in gravity "high" *A*) were obtained with a Bradley telemetered sonobuoy system. Range was determined from the water-wave velocity of 1.48 km/s measured on line *B*. All points on the travel-time graph are fitted by a straight line with inverse slope  $5.89 \pm 0.08$  km/s and intercept  $0.13 \pm 0.03$  s (Fig. 2), confirming that high density basement rock crops out at the seabed.

The 40 and 50 km profiles, lines *A* and *B*, are parallel to the strike of the steep gravity gradient and are contained within gravity "low" *E*. They are 4 km apart. Three first arrival phases are identified on each line. The results of fitting straight line segments to the travel-time plots (Figs. 3 and 4) are shown in Table 1.

Uncertainties are standard errors from the process of fitting straight lines to the data by least squares.

The time intercepts for segments *A2* and *B2* are not consistent with layer 2 cropping out at a depth of 140 m on the seabed. A lower velocity layer, layer 1, is required. With an assumed velocity of 1.7 km/s layer 1 would be 450 m thick and with 2.0 km/s it would be 600 m thick. The latter velocity has been assumed for this interpretation.

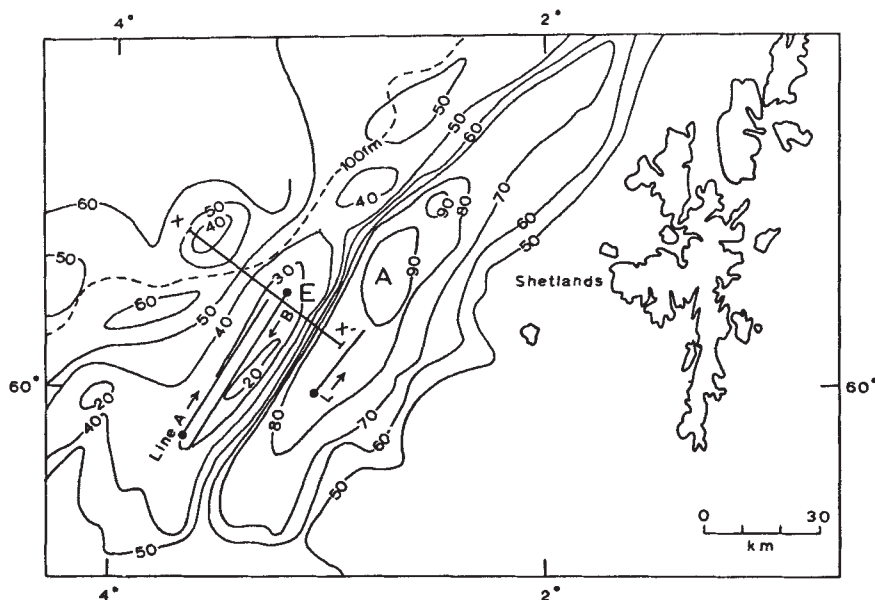


Fig. 1 Bouguer anomaly map showing locations of seismic refraction profiles (lines *A*, *B*, *L*) and the gravity profile *XX'*.



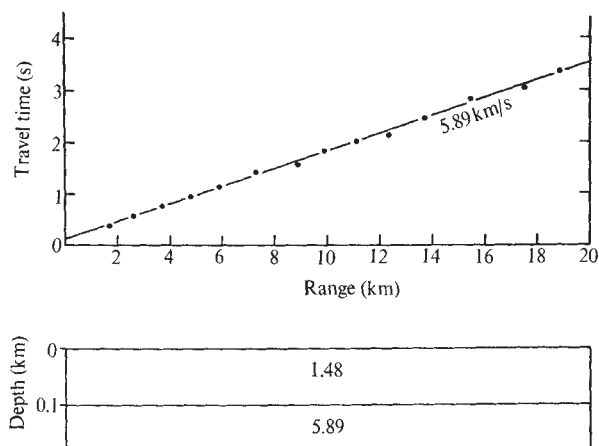


Fig. 2 Interpretation of the travel-time plot for line L.

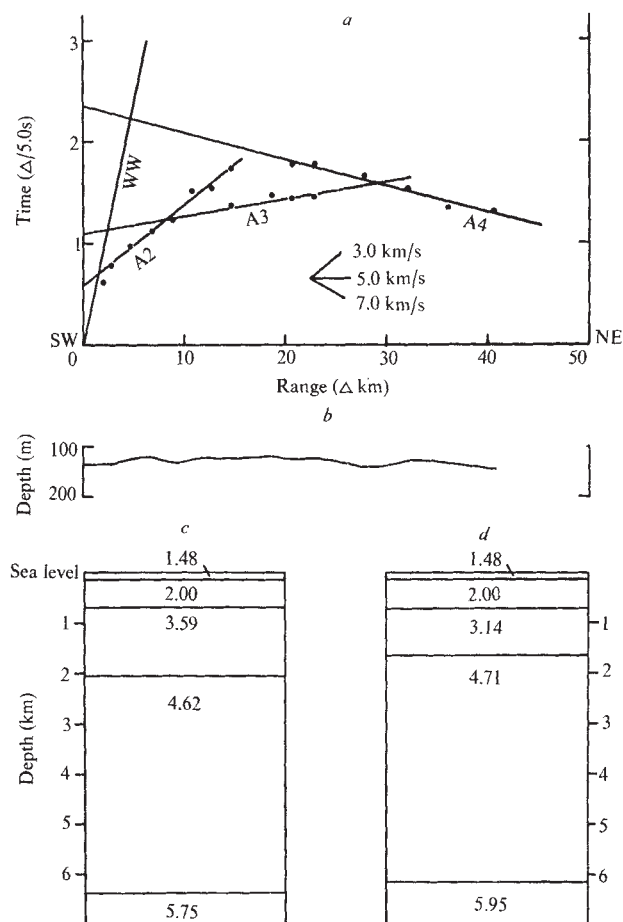


Fig. 3 a, Travel-time graph of seismic refraction profile A. b, Bathymetry along the profile. c, Interpreted model using observed apparent velocities. d, Model using mean velocities from profiles A and B. Layer velocities are in km/s.

There is close agreement between the apparent velocities of layer 3. The mean velocity of layer 4, 5.95 km/s, is correlated with the velocity of 5.89 km/s obtained for the crystalline basement on line L. The apparent velocities of 3.59 and 2.69 km/s obtained for layer 2 may reflect the presence of two layers or, more likely, the effects of dipping sediments and lateral variations (the regions in which the two determinations were made are separated by 40 km). The significantly lower time-intercepts for layers 3 and 4 on line A indicate that the upper boundary (and possibly the lower boundary) of layer 3 dips in a south-easterly direction towards the faulted margin of the basin.

Table 1 Fitting Straight Lines to Travel-time Plots

| Layer | Segment | Velocity (km/s)   | Time intercept (s) | No. of observations |
|-------|---------|-------------------|--------------------|---------------------|
| Water | WW      | $1.481 \pm 0.003$ |                    | 16                  |
| 2     | A2      | $3.59 \pm 0.09$   | $0.58 \pm 0.07$    | 6                   |
| 3     | A3      | $4.62 \pm 0.10$   | $1.10 \pm 0.08$    | 5                   |
| 4     | A4      | $5.75 \pm 0.08$   | $2.35 \pm 0.08$    | 6                   |
| 2     | B2      | $2.69 \pm 0.03$   | $0.53 \pm 0.02$    | 3                   |
| 3     | B3      | $4.80 \pm 0.04$   | $1.80 \pm 0.03$    | 5                   |
| 4     | B4      | $6.16 \pm 0.19$   | $2.98 \pm 0.19$    | 6                   |

Models are shown (Figs. 3 and 4) for both observed and mean velocities assuming horizontal layering along the profiles. The broad features of the structure indicated by this analysis are: (1) lower velocity sediments (layers 1 and 2) to a depth of 1.5 to 2 km beneath line A and 2.5 to 3 km beneath line B, (2) an intermediate layer with velocity about 4.7 km/s and (3) a crystalline basement at a depth of 6 to 7 km.

The gravity profile (XX', Fig. 1) of Bott and Watts<sup>1</sup> has been reinterpreted using controls imposed by the seismic refraction data (Fig. 5).

A steeply dipping boundary is required between sediments and basement on the south-eastern flank. A vertical fault has been assumed in both models. The maximum depth (3 km) of sediments beneath line B and a basement depth of 6.5 km have been used as constraints.

Two models are shown, one in which the western buried ridge is considered to be topography of the layer 3 surface and a second in which the high velocity (high density) basement comes up. The former model requires a lower density contrast between layer 3 and the basement with a correspondingly high

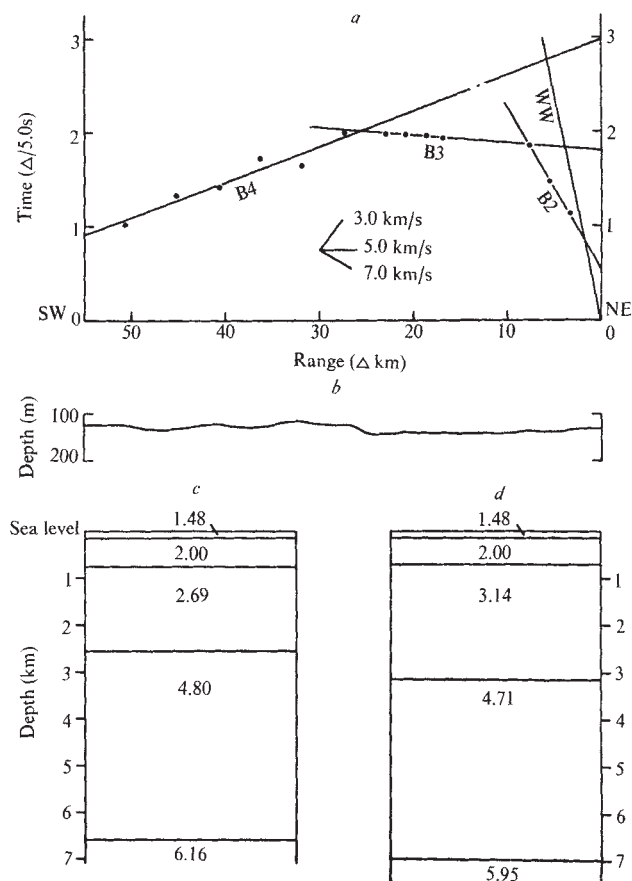


Fig. 4 a, Travel-time graph of seismic refraction profile B. b, Bathymetry along the profile. c, Interpreted model using observed apparent velocities. d, Model using mean velocities from profiles A and B. Layer velocities are in km/s.

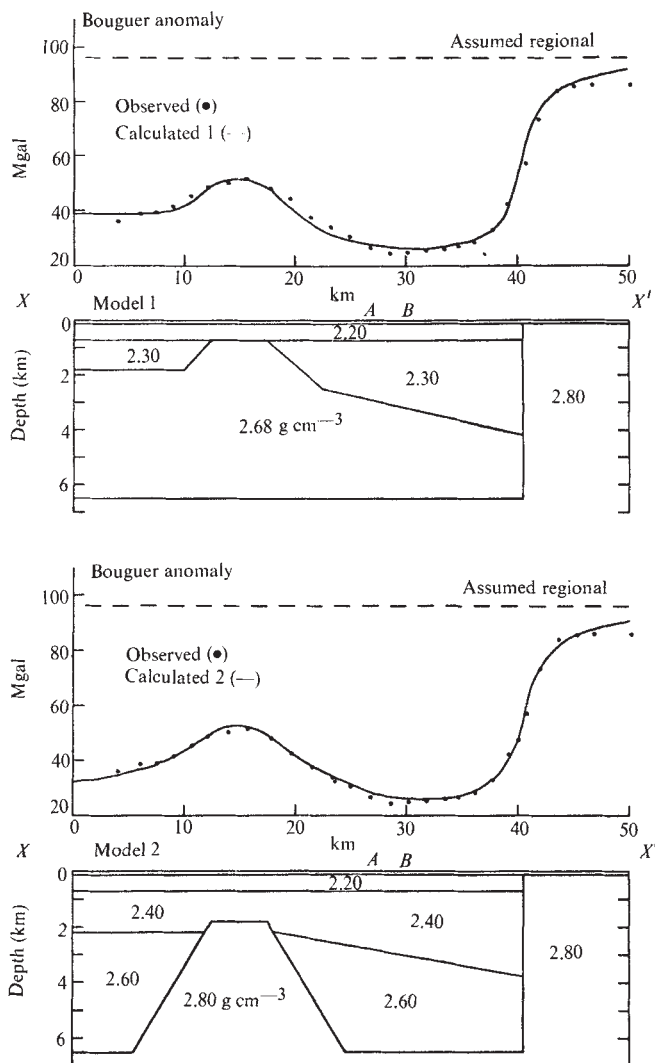


Fig. 5 Gravity profile across "low" E (profile XX', Fig. 1) showing the interpretation. Densities are linked to an assumed value of  $2.80 \text{ g cm}^{-3}$  for the basement.

contrast for layer 2. The densities shown are linked to an assumed basement density of  $2.80 \text{ g cm}^{-3}$  consistent with field measurements on Lewisian rocks<sup>3,4</sup>.

The necessary density contrasts and observed seismic velocities support the interpretation of Bott and Watts that the basin probably contains Mesozoic-Tertiary sediments. The maximum thickness of low density sediments is 3 to 4 km which is less than that obtained previously, the gravity field being satisfied with an additional negative contribution from layer 3.

The probable density of layer 3, 2.6 to  $2.7 \text{ g cm}^{-3}$ , and its velocity of 4.7 km/s are within the ranges found for Palaeozoic rocks and for Torridonian Sandstone<sup>3,5-7</sup>.

The structure of the basin is similar to that of the Cardigan Bay sedimentary basin. Both are probably fault-bounded and are structurally controlled along Caledonian trend directions. In Cardigan Bay, geophysical work<sup>8,9</sup> tied to the Mochras borehole<sup>10</sup> has established layers with seismic velocities of 2.3 and 3.5 km/s and the presence of Mesozoic and Tertiary sediments. The observed 70 mgal anomaly indicates an overall depth of 3.5–6.5 km. Neither the bottom of this basin nor a layer corresponding to the 4.7 km/s layer in the Shetland shelf basin has been detected by seismic methods.

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## Atmospheric Ice Nuclei from Decomposing Vegetation

THE sources and the composition of atmospheric ice nuclei (particles initiating the phase transition to ice at temperatures between  $0^\circ \text{C}$  and  $-40^\circ \text{C}$ ) are still largely unidentified, chiefly because they make up an extremely small fraction (perhaps 1 in  $10^6$ ) of the total atmospheric aerosol and because they are a heterogeneous mixture of substances whose only common property is the ability to nucleate ice.

Oceans and deserts do not seem to make a major contribution, but other naturally occurring substances, such as clays and various soils, have been shown to be moderately good ice nucleators, and extraterrestrial sources may also contribute<sup>1-3</sup>. Various compounds, such as silver iodide and leucine, are effective ice nuclei in the laboratory but their atmospheric abundances are thought to be small. On the whole, no major sources of atmospheric ice nuclei have been identified and it seems especially difficult to explain the presence of nuclei acting in the temperature range between  $0^\circ \text{C}$  and  $-10^\circ \text{C}$ .

Recent tests with derivatives of decaying vegetation suggest that a large source of active atmospheric ice nuclei might have

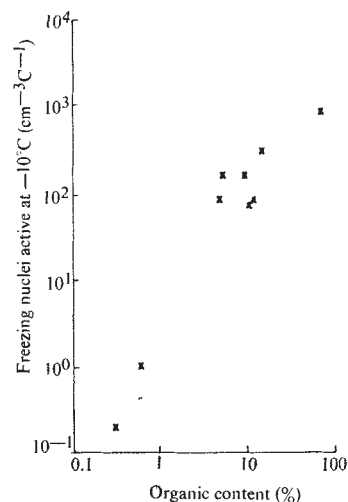


Fig. 1 Correlation of freezing nucleus concentrations with organic contents for sils of different origins.



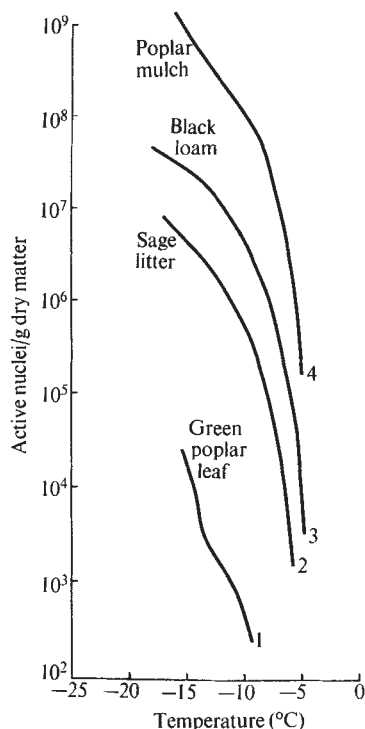


Fig. 2 Freezing nucleus spectra of four different sources. The numbers of nuclei as well as the warmest temperatures of activity increase in going from green leaves (1) to actively decomposing leaves (4).

been discovered. The starting point for this research was the finding<sup>4</sup> that soils having higher contents of organic matter are better nucleators than pure clays or sands. Soil samples from near the surface were found to contain more ice nuclei than samples from a few feet below the surface. Fig. 1 shows the correlation between freezing nucleus content and organic content for a selection of soil samples. Freezing nucleus contents were determined by the method of Vali<sup>5</sup>; the total organic contents were established by the methods of Peach *et al.*<sup>6</sup>.

Because the organic contents of soils derive principally from decomposed vegetation, we attempted to trace the evolution of this matter with respect to nucleating ability. Samples were collected from different parts of trees and shrubs and at different times of the year, as well as from old litters that had been on the ground for one or more years. In addition, some leaves were collected, masticated, allowed to decay in the laboratory under either aerobic or anaerobic conditions and examined repeatedly during the decay process. Nucleus contents were determined in each case by immersing a small amount of lightly crushed matter in distilled water (1 part material to 100 parts of water). The solid content was then allowed to settle out and tests were made on the clear water above the sediment. After sedimentation the solid content of the water was estimated to be considerably less than 1 part

in  $10^4$  parts of water. Freezing nucleation tests were run on the University of Wyoming automated nucleus spectrometer.

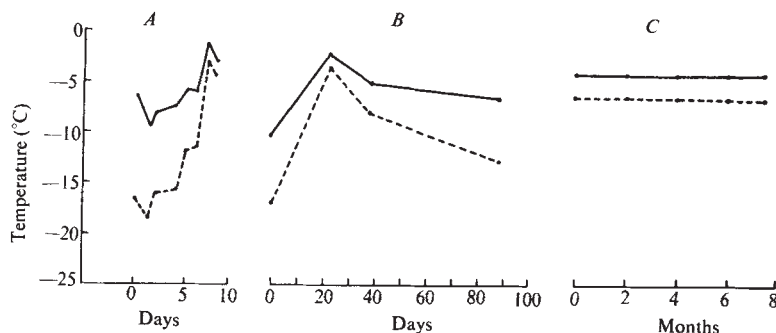
From the nucleation tests, it is evident that fresh green leaves or other parts of living plants do not provide large amounts of freezing nuclei. At the other end of the scale, the highest concentrations of nuclei were detected in poplar and spruce leaves undergoing active decomposition under aerobic conditions. Anaerobic decomposition leads to the development of considerably lower nucleus contents. Nucleus spectra for typical samples are shown in Fig. 2; it is apparent that the range of concentrations at  $-10^\circ\text{C}$ , for example, varies by as much as five orders of magnitude among these leaf samples, with the actively decomposing poplar mulch showing the highest activity. For comparison, the concentration of nuclei in purified clays, at  $-10^\circ\text{C}$ , is generally in the range  $10^2$  to  $10^4\text{ g}^{-1}$ . The results thus clearly indicate that decomposing vegetation is much superior to clays as a source of freezing nuclei and that the nucleating abilities of soils are therefore related to their organic contents and not to their basic mineral constituents. Hence, an explanation for the results shown in Fig. 1 is readily available. Pending closer identification of the active ingredients, the nuclei found in decomposing leaves were termed "leaf-derived nuclei" or LDN.

Perhaps the clearest demonstration that the LDN result from active decomposition emerged from experiments in which nucleus contents were examined at various times during the decay process. Fig. 3 illustrates the results of these experiments; the temperatures at which the nucleus concentrations were  $10$  and  $10^3\text{ cm}^{-3}$  are given for samples that had 1 part of solid to 100 parts of water. Brown and green poplar leaves both exhibited variations in activity during the decomposition process. Most significant is the occurrence of periods of very great activity, as evidenced by the high freezing temperatures of  $-1.3^\circ\text{C}$  on day 7 for the brown sample and  $-2.2^\circ\text{C}$  on day 22 for the green sample. The frequency of testing was insufficient in these experiments to pinpoint the changes in activity with greater detail. For nuclei derived from old well-decomposed litters, no change was apparent during a very long period of testing. The indication provided by these results is that the nuclei are by-products of active bacterial decomposition.

Can LDN contribute to atmospheric populations of ice nuclei? Mechanical contact between a clean surface and dry poplar litter was found to result in considerable numbers of active nuclei on the surface even though no visible residue was evident. Evaporation to dryness of water extracts has shown that essentially all activity remains in the dry residues. The materials constituting the LDN were also found to be volatile; heating of dry litters to temperatures somewhat in excess of  $100^\circ\text{C}$  and exposing a clean surface to these vapours resulted in high ice nucleation activity in the condensate on the surface. Hence, it appears that either mechanical lifting of aerosols (derived directly or by water extractions) or recondensation of vapours from the tree litters could lead to atmospheric ice nuclei.

These results refer mainly to litters from spruce and poplar trees. These are among the most abundant genera, especially

Fig. 3 Time variations of nucleus contents for poplar leaves which were brown (A) and green (B) when collected and for litter of earlier years (C). The points represent the temperatures at which the nucleus concentrations of water suspensions had indicated values. —,  $K=10\text{ cm}^{-3}$ ; ---,  $K=10^3\text{ cm}^{-3}$ .



at mid-latitudes, and were selected for testing for that reason. But work now in progress indicates that our findings are not restricted to the two species mentioned but are quite probably much more general in nature.

Decomposing vegetation is a common and extensive constituent of the surface coverage of the Earth. Because of the great abundance of nuclei that these materials provide, decomposing vegetation may indeed be an important source of atmospheric ice nuclei. The warm temperatures at which the LDN are active are remarkable because such very active nuclei are of pronounced importance for many precipitation processes. A further possibility is that LDN might become useful for artificial cloud seeding instead of silver iodide which is in common use today. The activities shown in Fig. 2 are about  $10^3$  lower than those typically reported for silver iodide seeding generators, but it must be recognized that the magnitudes given in Fig. 2 refer to the unrefined bulk leaf matter, of which only a minute fraction acts as freezing nuclei. By separation or by manufacturing (after identification) of the active ingredients, activities exceeding those of silver iodide are almost certain to be reached. The use of such natural substances for cloud seeding would be far preferable than the use of even mildly toxic heavy metals.

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## Lowering of the Saturation Solubility of Oxygen by the Presence of Another Gas

The solubility properties of gas mixtures dissolved in water have been much neglected in spite of the importance of the role of dissolved gases to a wide range of ecological and

physiological problems. It has been generally accepted that for a gas mixture the variation of the solubility of each component with pressure can be fairly well estimated using the Henry's law relationship. It is quite generally assumed that the presence of a second gas does not affect the saturation solubility value.

Our results do not support this assumption. Fig. 1 shows the saturation solubility diagram for an oxygen-nitrogen mixture. The data are measured at 25.00° C and at a total pressure of 1 atmosphere. The actual amount of gas dissolved in a 1 cm<sup>3</sup> sample of the saturated water was measured using a helium-stripping gas chromatographic technique. It is seen that the saturation solubility of each gas is depressed well below the expected Henry's law line. The lowering of the saturation solubility of oxygen is by 25% at  $P_{O_2}=0.25$ , 26% at  $P_{O_2}=0.5$  and 12% at  $P_{O_2}=0.75$ . A similar mutual lowering of saturation solubility is observed for  $O_2-CH_4$  and  $N_2-CH_4$  mixtures.

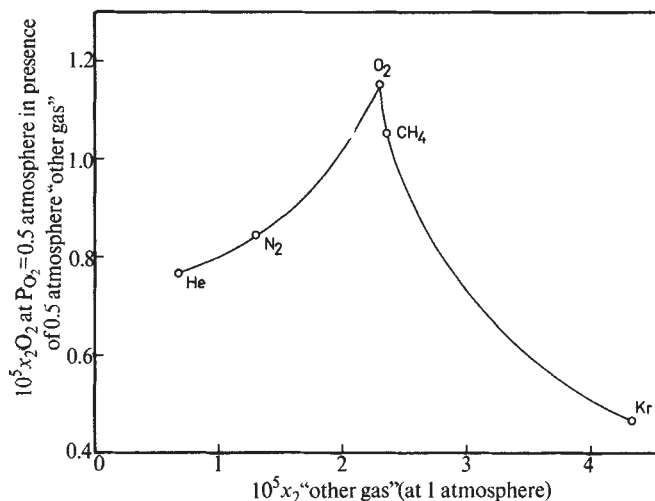


Fig. 2 Dependence of saturation solubility of oxygen (at 0.5 atmosphere partial pressure in mixture with "other" gas at 0.5 atmosphere partial pressure) on the saturation solubility of "other" gas (as pure gas at 1 atmosphere pressure). Data in water solvent at 25.00° C.

The depression of oxygen saturation solubility is of particular interest and Fig. 2 is a plot of the saturation solubility of oxygen at 0.5 atmosphere partial pressure, as a function of the saturation solubility of the second gas when dissolved in water as a pure gas at 1 atmosphere. Both gases more soluble than oxygen (saturation mol fraction solubility as pure gas at 1 atmosphere ( $\chi_2 = 2.31 \times 10^{-5}$ )) and gases less soluble than oxygen cause a lowering of oxygen saturation solubility. Thus, helium ( $\chi_2 = 0.68 \times 10^{-5}$ ) depresses the saturation solubility from the expected Henry's law value by 34% and krypton ( $\chi_2 = 4.32 \times 10^{-5}$ ) by 60%.

We emphasize that the reported data are for a total gas pressure of 1 atmosphere and at an oxygen partial pressure of 0.5 atmosphere. The inference of the effect of the "other gas" depressing oxygen saturation solubility as an alternative theory for non-reactive gas anaesthesia is worthy of note. Further studies at lower oxygen partial pressures and at higher pressures of the "other" gas are being made.

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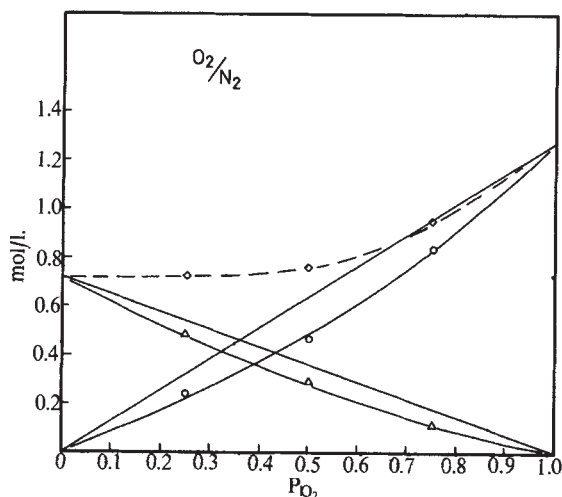


Fig. 1 Saturation solubility curves for  $O_2$  and  $N_2$  gas mixtures at various partial pressures of  $O_2$  at a total pressure of 1 atmosphere. Data at 25.00 ( $\pm 0.01$ )° C. Solvent, water.  $\circ$ , Oxygen solubility (mol/l.);  $\Delta$ , nitrogen solubility (mol/l.);  $\diamond$ , total mol/l. solubility.



## Origin of QSOs

In a recent issue of *Nature*<sup>1</sup> the opinion was expressed that the lack of observed blueshifts for quasars essentially rules out local origin. While this may be a valid objection to some forms of local theory, it is not so for the very local theory in which quasars were ejected a few million years ago from the centre of our Galaxy, the nearest being only a few hundred thousand light years away. Such a distance is consistent with the observed upper limits on proper motion<sup>2</sup>.

On this basis, local quasars would be no brighter than type O stars,  $10^4$  to  $10^5$  times the brightness of the Sun, and would have only redshifts. Of course such quasars, if ejected by other galaxies, would be expected to have blueshifts, but would be too dim to make a blueshift measurement easy even if ejected by relatively nearby galaxies. An attractive feature of this theory is that it can even account for radio galaxies in terms of ejection of clouds of quasars from gravitationally-collapsing galactic centres.

Because lack of blueshifts is not a valid objection to the very local model<sup>3-5</sup>, it is surprising that it continues to be cited as the crucial objection to local quasars. The very local quasar theory deserves more attention, especially in connexion with recent discoveries of very rapid relative motion of radio sources associated with several quasars<sup>6,7</sup>.

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## Non-Velocity Redshifts in Galaxies

A RECENT editorial in *Nature*<sup>1</sup> on non-velocity redshifts in galaxies overlooks an important early reference to the subject<sup>2</sup>. In that work we demonstrated from an analysis of all galaxy redshift data available at that time (1963) that (a) the Holmberg effect (a supposed systematic error depending on galaxy type, exposure time and nuclear magnitude) does not exist—that is, the effects noted by Holmberg were accidental and disappeared when a larger sample was considered; and (b) the mean redshift of the Virgo cluster galaxies increases systematically and monotonically from  $\langle V \rangle \simeq +900$  km s<sup>-1</sup> at type E to  $\langle V \rangle \simeq +1,800$  km s<sup>-1</sup> at type Sc, and more precisely as follows:

|             |                    |                                        |
|-------------|--------------------|----------------------------------------|
| Ellipticals | (E, $n=17$ ):      | $+900 \pm 120$ (km s <sup>-1</sup> )   |
| Lenticulars | (L, $n=20$ ):      | $+1,080 \pm 117$ (km s <sup>-1</sup> ) |
| Spirals     | (Sa-Sb, $n=10$ ):  | $+1,398 \pm 194$ (km s <sup>-1</sup> ) |
| Spirals     | (Sbc, Sc, $n=7$ ): | $+1,789 \pm 231$ (km s <sup>-1</sup> ) |

The difference  $\langle V(S) \rangle - \langle V(E, L) \rangle = (1,559 \pm 148) - (990 \pm 85) = +569 \pm 170$  is significant at the 3.3  $\sigma$  level.

This systematic difference was first reported by G. V.<sup>3</sup> in a study of the structure of the Virgo cluster in which it was suggested that the gross differences between the velocity distributions, as well as in the apparent distributions on the sphere of the E and L galaxies, on the one hand, S and I galaxies on the other, could be most simply interpreted by the assumption that the traditional Virgo "cluster" is actually the optical superposition of two clusters of different types, shapes and

compositions at different distances along the same line of sight. There are, however, equally good arguments supporting the concept of a simple cluster at a well defined mean distance<sup>4</sup>. If so, non-velocity shifts must be considered a real possibility.

We wish to caution, however, that small systematic differences (some tens of km s<sup>-1</sup>, although not hundreds as discussed above) can easily arise from errors in the rest wavelengths adopted for many of the broad lines (H, K) or blends (G band, and so on) used to measure redshifts of early-type galaxies (E, L) on low dispersion spectrograms and which may not be strictly consistent with the system defined by the emission lines (H $\alpha$ , N<sub>1</sub>, N<sub>2</sub>,  $\lambda 3727$ ) available in most spirals<sup>5,6</sup>.

Finally, we wish to point out that the initial discovery of large redshift differences in interconnected galaxies was made jointly by Zwicky, Minkowski and Humason nearly 20 years ago in their study of the famous triple system including IC 3481-3483 (see ref. 7).

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## Tritium in Deuterated Compounds

IN the past few years deuterated compounds have become readily available and are in wide use, especially as solvents for NMR spectroscopy. D. Arigoni has informed us (personal communication) that heavy water (D<sub>2</sub>O) can contain appreciable quantities of tritium. It has been known for some time that tritium is present in D<sub>2</sub>O obtained as a residue after the prolonged electrolysis of natural water<sup>1</sup>. But most users of deuterium compounds seem to be unaware of the presence of tritium, often in amounts which require a licence for possession. Those using tritium as a tracer should be cautioned regarding the potential contamination resulting from the use of D<sub>2</sub>O and other deuterated compounds. We have measured the tritium content of several commercial deuterated compounds which were in routine use in our laboratory, and the results are summarized in Table 1. All the compounds had a nominal deuterium content > 99%.

**Table 1** Tritium Content measured by Liquid Scintillation Counting

| Compound                            | Commercial source         | <sup>3</sup> H activity<br>( $\mu\text{Ci mol}^{-1}$ ) |
|-------------------------------------|---------------------------|--------------------------------------------------------|
| D <sub>2</sub> O                    | Aldrich Chemical Co.      | 1.6                                                    |
| D <sub>2</sub> O                    | Bio-Rad Laboratories      | 0.00012                                                |
| D <sub>2</sub> O                    | Norell Chemical Co.       | 1.4                                                    |
| D <sub>2</sub> O                    | Stohler Isotope Chemicals | 1.8                                                    |
| Acetic acid-d <sub>4</sub>          | Stohler Isotope Chemicals | 7.9                                                    |
| Acetone-d <sub>6</sub>              | Aldrich Chemical Co.      | 3.6                                                    |
| Benzene-d <sub>6</sub>              | Diaprep Inc.              | 15.4                                                   |
| Chloroform-d                        | Aldrich Chemical Co.      | 0.51                                                   |
| Dimethylsulphoxide-d <sub>6</sub>   | Stohler Isotope Chemicals | 6.1                                                    |
| Methanol-d <sub>4</sub>             | Prochem Ltd               | 0.079                                                  |
| Nitromethane-d <sub>3</sub>         | Stohler Isotope Chemicals | 1.6                                                    |
| Pyridine-d <sub>5</sub>             | Prochem Ltd               | 9.1                                                    |
| Sodium borodeuteride-d <sub>4</sub> | Stohler Isotope Chemicals | 7.5                                                    |

The tritium content of three of the samples of  $D_2O$  was almost the same, suggesting that they were all obtained from the same source. On the other hand, the sample obtained from Bio-Rad Laboratories had very low activity ( $<15$  disintegrations  $\text{min}^{-1} \text{ml}^{-1}$ ).

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## BIOLOGICAL SCIENCES

### Role of Ribosomal Subunits in Eukaryotic Protein Chain Initiation

WE report here the isolation from messenger-free 80S wheat embryo ribosomes of 40S and 60S ribosomal subunits active in protein synthesis directed by natural messenger RNA (Table 1). This has enabled us to provide firm biochemical evidence satisfying the hypothesis that initiation in eukaryotic cells proceeds through the formation of a 40S ribosomal subunit-mRNA-Met-tRNA complex (complex I) which subsequently combines with a 60S ribosomal subunit to form an 80S monoribosome (complex II) functional in protein synthesis.

In previous investigations, portions of the total sequence presented here have been explored. Heywood and Thompson<sup>1</sup> have shown attachment of <sup>32</sup>P-labelled myosin mRNA to the 40S subunit component of unfractionated salt-washed ribosomes by sucrose gradient analysis. This binding required initiation factor EF<sub>3</sub> and GTP. Addition of unfractionated

**Table 1** TMV-RNA Directed <sup>14</sup>C-Amino-acid Incorporation by Isolated Wheat Embryo Ribosomal Subunits

|                       | <sup>14</sup> C-Amino-acid incorporation (pmol) |
|-----------------------|-------------------------------------------------|
| 40S                   | 0.17                                            |
| 60S                   | 0.27                                            |
| 40S + 60S             | 7.63                                            |
| 40S + 60S (– TMV-RNA) | 0.36                                            |

Incubation of 0.4 ml. final volume contained: 25 mM Tris-acetate, pH 8.1, 1.0 mM ATP, 8 mM creatine phosphate, 16  $\mu$ g creatine phosphokinase, 25  $\mu$ M GTP, 10  $\mu$ g TMV-RNA, 2.3 mM dithiothreitol, 90 pmol 8-<sup>14</sup>C-aminoacyl-tRNAs (1 pmol=300 c.p.m.), 3.6 mM MgAc<sub>2</sub>, 45 mM KCl, 0.16 ml. of subunit-free S-100 (see below) and 0.3 A<sub>260</sub> units of 40S subunit and/or 0.6 A<sub>260</sub> units 60S subunits. (This amount of subunits was used for all experiments reported here. Molar ratio of 1 : 1 was optimal for subunit activity.) After incubation for 30 min at 30° C, hot TCA-insoluble radioactivity was determined<sup>7</sup>. Subunit-free S-100 was obtained by layering 1.0 ml. S-100<sup>8</sup> over a 4 ml. 15–25% linear sucrose gradient containing 100 mM KCl, 2 mM MgAc<sub>2</sub>, 2 mM Tris-acetate, pH 7.6, and 3 mM mercaptoethanol, centrifuging in an SW50.1 rotor at 50,000 r.p.m. for 80 min, and then removing the upper 1.0 ml.

Ribosomal subunits were prepared from dry wheat embryo ribosomes. Approximately 40 A<sub>260</sub> units of ribosomes in 0.5 ml. of 40 mM KCl, 0.33 mM MgAc<sub>2</sub>, 1 mM Tris-acetate, pH 7.6, 1 mM mercaptoethanol and 7% glycerol were layered on a 10–30% hyperbolic sucrose gradient containing 40 mM KCl, 0.08 mM MgAc<sub>2</sub>, 2 mM Tris-acetate, pH 7.6 and 3 mM mercaptoethanol. Following centrifugation in an SW27 rotor for 18 h at 27,000 r.p.m. the gradients were scanned for absorbance at 254 and peak fractions of each subunit (3.3 ml.) collected. The two subunit fractions were made 1 mM with respect to MgAc<sub>2</sub> and pelleted by centrifugation in a Ti 50 rotor at 50,000 r.p.m. for 270 min. Pellets were resuspended and stored at –20° C in a solution of 20% glycerol, 20 mM KCl, 1 mM MgAc<sub>2</sub>, 4 mM mercaptoethanol and 2 mM Tris-acetate, pH 7.3.

initiation factors and tRNA resulted in the cosedimentation of a portion of the labelled mRNA with the 75S monoribosome. Pragnell *et al.*<sup>2</sup> have made similar observations in the reticulocyte system. In this case, however, binding of the globin mRNA required that it be present as a ribonucleoprotein and not as free 9S RNA. The reticulocyte and plasmacytoma systems have also yielded 40S subunits which have been used to study the AUG trinucleotide-dependent binding of Met-tRNA<sub>f</sub><sup>3,4</sup>. Anderson and co-workers<sup>5,6</sup> have further indicated that, when 40S subunits bind <sup>35</sup>S-Met-tRNA in the presence of 60S subunits, a portion of the radioactivity can be found associated with monoribosomes.

The data of Table 1 confirm that 40S or 60S subunits alone are inactive in protein synthesis. Combined, however, these subunits obtained by low salt dissociation of 80S ribosomes are quite active in amino-acid incorporation (see comparison with 80S ribosomes below). The following data illustrate the sequential role of each subunit in chain initiation. Attachment of mRNA (in this case TMV-RNA) takes place on the 40S subunit as determined by the ability of these two components to direct the specific binding of Met-tRNA (Table 2). 60S subunits do not bind Met-tRNA nor do they enhance binding to 40S subunits. The conditions of incubation and requirements for Met-tRNA binding to 40S subunits are identical to those established previously for 80S ribosomes<sup>9</sup>. That is, in addition to initiation factors C and D, the reaction requires the ribosomal subunit, ATP and GTP (GMP-PCP partially substitutes for GTP). The 40S subunit reaction is specific for Met-tRNA and, as with 80S ribosome Met-tRNA binding<sup>9</sup>, the initiator species, Met-tRNA<sub>i</sub>, is bound in preference to Met-tRNA<sub>m</sub> (the species which inserts internal methionine).

**Table 2** TMV-RNA Directed Binding of Met-tRNA to Ribosomal Subunits

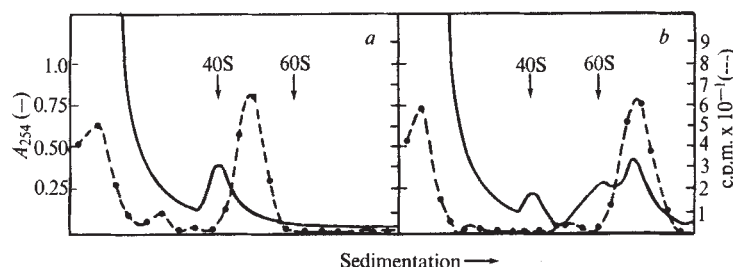
|                                    |           | Met-tRNA bound (pmol) |           |          |
|------------------------------------|-----------|-----------------------|-----------|----------|
|                                    |           | + TMV-RNA             | – TMV-RNA | $\Delta$ |
| Met-tRNA                           | 40S       | 1.09                  | 0.28      | 0.81     |
| Met-tRNA                           | 60S       | 0.07                  | 0.08      | 0        |
| Met-tRNA                           | 40S + 60S | 1.00                  | 0.24      | 0.76     |
| Met-tRNA <sub>i</sub>              | 40S       | 0.64                  | 0.23      | 0.41     |
| Met-tRNA <sub>m</sub>              | 40S       | 0.18                  | 0.05      | 0.13     |
| 8- <sup>14</sup> C-aminoacyl tRNAs | 40S       | 0.25                  | 0.25      | 0        |

The reaction mixture in a total volume of 0.34 ml. contained: 1.1 mM methionine, 30 mM Tris-acetate, pH 8, 1.1 mM ATP, 25  $\mu$ M GTP, 10  $\mu$ g TMV-RNA, 2.6 mM dithiothreitol, 1.3 mM MgAc<sub>2</sub>, 51 mM KCl, 0.3 A<sub>260</sub> units 40S subunits and/or 0.6 A<sub>260</sub> units 60S subunits, initiation factors ["C" (step 1–0.64 mg) and "D" (step 2–0.11 mg)]<sup>9</sup>, and 38 pmol unresolved <sup>14</sup>C-Met-tRNA (1 pmol = 330 c.p.m.) or 34 pmol <sup>35</sup>S-Met-tRNA<sub>i</sub> or <sup>35</sup>S-Met-tRNA<sub>m</sub> (1 pmol = 5,850 c.p.m.) or 51 pmol 8-<sup>14</sup>C-aminoacyl-tRNAs (containing <sup>14</sup>C-labelled leucine, serine, valine, lysine, threonine, proline, phenylalanine and glutamic acid). After incubation for 10 min at 20° C, radioactivity retained on nitrocellulose filters was determined<sup>9</sup>.

The selective binding of Met-tRNA offers strong presumptive evidence that a 40S ribosomal subunit-mRNA-Met-tRNA complex is an intermediate in chain initiation. Validation of that presumption, however, requires that the complex is capable of combining with 60S subunits to form a monoribosome complex which in turn can support ongoing peptide synthesis. The data of Table 3 provide direct evidence for this. Here 40S subunits are incubated under conditions similar to those used for Met-tRNA binding. Next, aurintricarboxylic acid (ATA) is added (to block mRNA attachment<sup>10</sup> and thus prevent further formation of complex I) along with 60S subunits and the incubation is resumed under conditions favourable to peptide synthesis. It is clear that the complex formed in the presence of 40S subunits is functional, and requires 60S subunits for subsequent peptide synthesis. 60S subunits may be supplied as such or from the equilibrium dissociation of 80S ribosomes. In the latter case, the level of amino-acid



**Fig. 1** Sucrose gradient analysis of the sequential formation of (a) initiation complex I, and (b) initiation complex II. Met-tRNA was bound to 40S subunits in incubations similar to those of the binding assay of Table 2 with the following modifications: 6  $\mu$ g STNV-RNA (the RNA of satellite tobacco necrosis virus) replaced TMV-RNA; and a subsequent 3 min at 20°C incubation was carried out to which was added in 0.06 ml., 0.92  $\mu$ mol of MgAc<sub>2</sub> and, in (b), 0.6  $A_{260}$  units of 60S subunits. The reaction products were layered on a 7.5–25% linear sucrose gradient containing 40 mM KCl, 1 mM MgAc<sub>2</sub>, 2 mM Tris-acetate, pH 7.6, and 3 mM mercaptoethanol. Following centrifugation in an SW50.1 rotor for 80 min at 50,000 r.p.m., the gradients were scanned for absorbancy at 254 nm and divided into 20 fractions. Each fraction was analysed for radioactivity retainable on nitrocellulose filters as in the binding assay of Table 2.



incorporation obtained with isolated 40S subunits corresponds well with that obtained with an equivalent amount of 80S ribosomes (0.9  $A_{260}$  units containing approximately 0.3  $A_{260}$  units of 40S subunits) added to the preliminary incubation. The formation of a functional 40S subunit initiation complex apparently neither requires, nor is aided by, the presence of the 60S subunit.

**Table 3** Formation of Functional Initiation Complex I with 40S Ribosomal Subunits

| Preliminary incubation | Incorporation incubation (with ATA) | Amino-acid incorporation (pmol) |
|------------------------|-------------------------------------|---------------------------------|
| 40S                    | 60S                                 | 2.8                             |
| —                      | 60S                                 | 0.2                             |
| 60S                    | 40S                                 | 0.2                             |
| 40S                    | —                                   | 0.4                             |
| 40S + 60S              | —                                   | 3.2                             |
| 40S                    | 80S                                 | 5.7                             |
| —                      | 80S                                 | 0.4                             |
| 80S                    | —                                   | 6.0                             |

A preliminary incubation containing a volume of 0.33 ml., 25 mM Tris-acetate, pH 8.1, 1 mM ATP, 8 mM creatine phosphate, 16  $\mu$ g creatine phosphokinase, 25  $\mu$ M GTP, 10  $\mu$ g TMV-RNA, 2.3 mM dithiothreitol, 2.5 mM MgAc<sub>2</sub>, 51 mM KCl, 90 pmol 8-<sup>14</sup>C-aminoacyl-tRNAs (1 pmol=300 c.p.m.), initiation factors ["C" (step 1–0.64 mg) and "D" (step 1–0.51 mg)]<sup>8</sup>, and 0.3  $A_{260}$  units 40S subunits and/or 0.6  $A_{260}$  units 60S subunits or 0.9  $A_{260}$  units of 80S ribosomes was carried out for 7 min at 20°. Thereafter, 1.6 nmol ATA (final concentration 40  $\mu$ M), 0.6  $\mu$ mol MgAc<sub>2</sub>, 0.03 ml. subunit-free S100 and either 40S subunits (0.3  $A_{260}$  units), 60S subunits (0.6  $A_{260}$  units) or 80S ribosomes (5.0  $A_{260}$  units) were added to a final volume of 0.40 ml. Following a 9 min incubation at 30°, the radioactive material insoluble in hot TCA was determined<sup>7</sup>.

The sequential reactions leading to the formation of initiation complexes I and II can be readily visualized by sucrose gradient analysis. In the incubation represented in Fig. 1a, 40S subunits, STNV-RNA (the monocistronic mRNA from satellite tobacco necrosis virus), and <sup>35</sup>S-Met-tRNA have joined to form complex I which has a sedimentation value of approximately 50S. If complex I is supplied with 60S subunits in an ensuing incubation there is a complete shift of labelled Met-tRNA into the monoribosome region of the gradient (Fig. 1b). Chromatographic analysis (not shown) of the <sup>35</sup>S radioactivity from the monoribosome peak shows that only free methionine is present and therefore that peptide bond formation is not required for monoribosome formation. These monoribosomal complexes are not, however, simply non-specific aggregates of complex I and 60S subunits. Recent experiments (to be published) have shown that two inhibitors of peptide chain initiation, 2-(4-methyl-2,6-dinitroaniline)-N-methyl propionamide (MDMP) and KF (neither of which interferes with messenger RNA directed binding of Met-tRNA<sub>i</sub> to ribosomes, but completely prevent its release by puromycin), specifically block the interaction of complex I and 60S subunits to form complex II. The possibility of conformational changes<sup>11,12</sup> associated with the formation of these complexes is under investigation.

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## Mechanism of Immunologically Specific Killing of Tumour Cells by Macrophages

THE increase in anti-microbial activity of macrophages which occurs in animals that have been infected with living micro-organisms is frequently non-specific<sup>1</sup>. In contrast, the anti-tumour activity of macrophages from immunized animals has been demonstrated *in vitro* to be immunologically specific<sup>2,3</sup>. The experiments to be described may throw light on this paradox. They show that the killing of tumour cells by macrophages is made up of an immunologically specific interaction which is followed by a non-specific lethal reaction. A similar pattern has been described for the bactericidal action of immune macrophages<sup>3</sup>. We have described three ways of obtaining immunologically specific macrophages cytotoxic to tumour cells<sup>3,5</sup>. These are (1) from the peritoneal cavity of suitably immunized mice (see later); (2) "arming" *in vitro* by contact of non-immune macrophages with spleen cells from hyperimmunized mice, and (3) "arming" *in vitro* by exposure of non-immune macrophages to the cell-free supernatant obtained when spleen cells from immunized mice

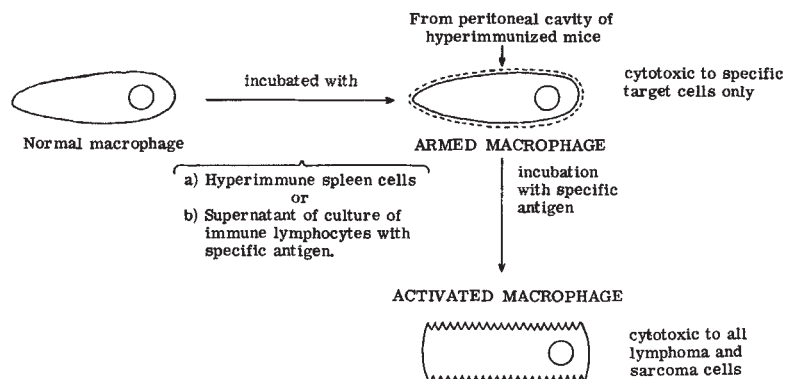


Fig. 1 Mechanism of macrophage cytotoxicity.

are cultured with the specific antigen. All such macrophages, on coming into contact with specific antigens, undergo a transformation (referred to as "activation") which renders them capable of killing. The actual destruction of the target cell following direct contact with the "activated" macrophages is non-specific. Moreover, this non-specific killing may also be demonstrated by macrophages "armed" against tubercle bacilli (BCG) which are "activated" after exposure to the solubilized protein derivative from tubercle bacilli, PPD, and are able to kill tumour cells. In the original experiments<sup>3,5</sup> in which target lymphoma cells were added to "armed" macrophages the immunologically specific stage and the non-specific stage of the cytotoxic reactions were not separated and occurred sequentially (Fig. 1).

The procedure used to determine the cytotoxicity of macrophages was to observe the growth of target murine lymphoma cells when added to confluent macrophage monolayers in Petri dishes. As shown previously<sup>3</sup>, the target cells are killed as a result of membrane contact with the macrophages, and phagocytosis is a late event which occurs only after the lymphoma cells have become non-viable and are disintegrating. Immune macrophages were obtained from CBA mice which were immunized at 10 day intervals with two intraperitoneal injections of  $5 \times 10^6$  SL2 (DBA/2) or TLX9 (C57Bl) lymphoma cells or 0.1 ml. of percutaneous BCG vaccine (Glaxo) originally suspended in 1.0 ml. of saline. Macrophages were harvested from the peritoneal cavity 10 days after the second immunization, and  $2 \times 10^6$  in Fischer's medium<sup>6</sup> supplemented with 10% heat-inactivated foetal bovine serum were seeded into 3.5 cm Falcon dishes to give a confluent monolayer. Armed macrophages were obtained by adding  $5 \times 10^6$  spleen cells from CBA mice hyperimmunized as above 10 days previously to non-immune CBA macrophage monolayers and incubating for 24 h, followed by thorough washing until non-adherent cells such as lymphocytes could not be detected by microscopic examination. Using these immune, armed and non-immune

macrophage cultures the immunological specificity of the cytotoxicity and also the non-specific stage before and after exposure to the specific or non-related antigen were analysed. To render cytotoxic macrophages non-specific in their killing, monolayers were exposed to their specific antigen, either to  $10^6$  lymphoma cells or  $10 \mu\text{g}$  PPD  $\text{ml}^{-1}$  for 4 h. They were then washed thoroughly and challenged with either  $10^5$  SL2 or TLX9 cells in 3 ml. of growth medium and left for 48 h when viable lymphoma cells were counted. These lymphomas showed no cross-immunity, that is, CBA mice immunized with the SL2 tumour did not have antibodies, lymphocytes or macrophages directed against TLX9 cells or vice versa. Both SL2 and TLX9 cells grow from less than 100 cells in the syngeneic host, but are rejected—following a period of growth—in allogeneic CBA mice when an inoculum of  $5 \times 10^6$  cells is used. Both lymphomas grow readily in tissue culture (Fig. 2) using Fischer's medium + 10% foetal calf serum.

Table 1 shows that the cytotoxicity of macrophages taken from immunized mice 10 days after the second immunization is immunologically specific, that is, macrophages from mice immunized against SL2 cells will not kill TLX9 cells and vice versa, while those from mice immunized with BCG are not cytotoxic to either SL2 or TLX9 cells. After the macrophages from immunized mice have been exposed to the specific antigen (but not if they are exposed to a non-related antigen), however, they acquire the capacity to kill both SL2 and TLX9 cells.

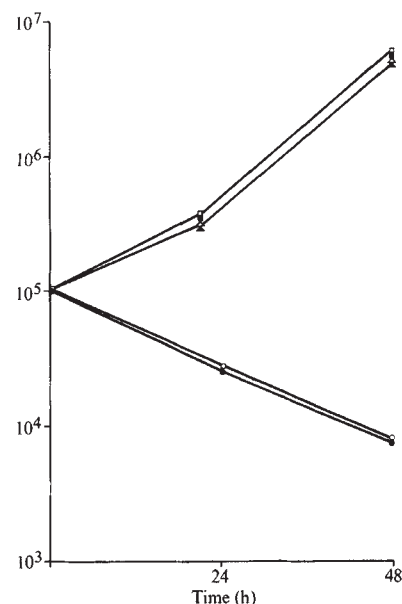


Fig. 2 Effect of immune macrophage monolayers on the growth of allogeneic lymphoma cells in culture. □, SL2 on non-immune CBA macrophages; ■, SL2 on macrophages immune against TLX9 cells; ○, SL2 on macrophages immune against SL2 cells; △, TLX9 on non-immune CBA macrophages; ▲, TLX9 on macrophages immune against SL2 cells; ●, TLX9 on macrophages immune against TLX9 cells.

Table 1 Cytotoxicity of Macrophages from Immunized Mice

| CBA mice immunized against: | Macrophages incubated with: | Cytotoxicity* at 48 h to: |          |
|-----------------------------|-----------------------------|---------------------------|----------|
|                             |                             | SL2 (%)                   | TLX9 (%) |
| SL2 lymphoma (DBA/2)        | No antigen                  | 99                        | <10      |
| TLX9 lymphoma (C57Bl)       | No antigen                  | <10                       | 99       |
| BCG                         | No antigen                  | <10                       | <10      |
| SL2 lymphoma                | SL2 cells                   | 99                        | 79       |
|                             | TLX9                        | 99                        | <10      |
|                             | PPD                         | 99                        | <10      |
| TLX9 lymphoma               | TLX9 cells                  | 75                        | 99       |
|                             | SL2 cells                   | <10                       | 99       |
|                             | PPD                         | <10                       | 99       |
| BCG                         | PPD                         | 92                        | 84       |
|                             | SL2 cells                   | <10                       | <10      |
|                             | TLX9 cells                  | <10                       | <10      |

\* Cytotoxicity expressed as percentage growth inhibition in relation to growth in normal or control CBA cultures.



Table 1 also shows that the antigenic stimulus required to produce "activated" macrophages capable of killing non-specific cells was not confined to the allogeneic lymphoma cells but could be brought about by BCG organisms.

An identical pattern of behaviour was shown by macrophages which had been "armed" *in vitro* with spleen cells from immunized mice (Table 2). The cytotoxicity of these "armed" macrophages was directed only against the cell used for immunization, but they could be "activated" by contact with specific antigen, after which both SL2 and TLX9 cells were killed in a non-specific manner.

**Table 2** Cytotoxic Activity of Macrophages from Non-immune CBA Mice "armed" *in vitro* by Incubation with Spleen Cells from Immunized Mice

| Macrophages incubated with spleen cells from mice immunized against: | Armed macrophages incubated with: | Cytotoxicity at 48 h to: |          |
|----------------------------------------------------------------------|-----------------------------------|--------------------------|----------|
|                                                                      |                                   | SL2 (%)                  | TLX9 (%) |
| SL2 lymphoma                                                         | No antigen                        | 85                       | < 10     |
| TLX9 lymphoma                                                        | No antigen                        | < 10                     | 82       |
| BCG                                                                  | No antigen                        | < 10                     | < 10     |
| SL2 lymphoma                                                         | SL2 cells                         | 90                       | 78       |
|                                                                      | TLX9 cells                        | 83                       | < 10     |
|                                                                      | PPD                               | 86                       | < 10     |
|                                                                      | TLX9 cells                        | 70                       | 82       |
| TLX9 lymphoma                                                        | SL2 cells                         | < 10                     | 88       |
|                                                                      | PPD                               | < 10                     | 80       |
|                                                                      | TLX9 cells                        | < 10                     | < 10     |
| BCG                                                                  | PPD                               | 83                       | 88       |
|                                                                      | SL2 cells                         | < 10                     | < 10     |
|                                                                      | TLX9 cells                        | < 10                     | < 10     |

"Activation" of macrophages can also be achieved by a means other than that described here when interaction of immunologically "armed" macrophages with specific antigen is required. We have recently demonstrated that by exposing non-immune macrophages to very small amounts of double-stranded RNA, endotoxin or lipid A the macrophages will kill non-specifically<sup>7</sup>. These two methods of macrophage "activation" bear a close resemblance to mast cell degranulation which can be induced in an immunologically specific way or pharmacologically<sup>8</sup>.

Macrophages derived from immunized animals will only show the immunologically specific cytotoxicity if several days have elapsed between the last injection of immunizing cells and the collection of the peritoneal cells. If the macrophages are collected too soon after the immunization they may be "activated" (by contact with antigen *in vivo*) and the resultant macrophage monolayer will be non-specifically cytotoxic. In the experiments summarized in Tables 1 and 2 the peritoneal cells were taken 10 days after the second immunization and their cytotoxicity was completely specific. If a further dose of antigen was given, peritoneal macrophages collected 24–72 h later killed both SL2 and TLX9 cells whether they came from mice immunized with BCG, SL2 or TLX9 cells. Ten days after this third immunization, however, the "activated" macrophages had disappeared and the macrophages then resident in the peritoneal cavity exerted the immunologically specific cytotoxic reaction described above. These *in vivo* observations may explain why mice which have been injected with BCG organisms show an increased resistance to a subsequent tumour challenge<sup>8</sup>.

This investigation was supported by institutional grants from the Medical Research Council and the Cancer Research Campaign.

*Note added in proof.* In these and earlier experiments<sup>3,5,7</sup> monolayers of macrophages from non-immunized mice did not inhibit growth of the target cells. Recently, however, we have encountered non-specific growth inhibition by macrophages taken from C57/Bl and DBA/2 mice. This non-specific cytotoxicity is weak and usually is only manifested when target cells have been incubated with the macrophages for longer than 24 h. Our experiments indicate that this

non-specific cytotoxicity is associated with infection of our mouse colony by a persisting bacterium, *Pasteurella pneumotropica*, and perhaps others. We interpret this phenomenon as being due to macrophage activation which has occurred because the macrophages armed against the infecting organisms become activated, as indicated in the text. In a recent paper in *Nature New Biology* (235, 48; 1972), Hibbs, Lambert and Remington reported that following infection of mice with *Toxoplasma*, their macrophages killed mouse L cells. In this case also a persisting infection could give rise to activated macrophages by the mechanism described in the present paper.

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## Determination of the Effect of Feeding on Drinking by a Stochastic Identification Technique

TRANSFER functions and impulse responses are used by engineers as a means of describing, in a single equation, the relationship between input and output of a system. Frequency analysis, which uses sinusoidal input functions, is a classic method of determining transfer functions in physical systems, and has also been used for behavioural systems<sup>1</sup>. In the behavioural context, sinusoidal input functions are not always convenient, and the determination of the impulse response by application of a binary stimulus offers an alternative method of obtaining the same information as that gained by frequency analysis. In this report we describe a method by which the effect of feeding on drinking behaviour is assessed in terms of an impulse response. The method involves minimal interference with the natural course of behaviour, and we therefore hope that it will prove to be useful in a wide variety of behavioural situations.

In general, the method consists in repeatedly presenting a stimulus at random intervals, and correlating some aspect of the animal's behaviour (response function) with the stimulation sequence (input function). The response  $y(t)$  of a linear system to an input function  $x(t)$  can be given by the convolution integral,

$$y(t) = \int_{-\infty}^t x(u)h(t-u)du$$

where  $x(u)$  is the input at any time  $u$ , and  $h(u)$  the corresponding impulse response. When  $\tau = t - u$ ,

$$y(t) = \int_0^{\infty} h(\tau)x(t-\tau)d\tau$$

For a response  $y_k$  sampled at intervals  $T$

$$y_k = \int_0^T h(\tau)x(kT-\tau)d\tau + \int_T^{2T} h(\tau)x(kT-\tau)d\tau + \dots$$

$$= \int_{rT}^{(r+1)T} h(\tau)x(kT-\tau)d\tau$$

When  $x(kT - \tau)$  is held constant between  $rT$  and  $(r+1)T$ , it can be shown that

$$y_k = \sum_{s=1}^{\infty} W_s u_{k-s}$$

where the weighting sequence has value  $W_s$  at time  $s$  on the impulse response  $h(u)$ ,  $u$  is the sequence of sampled inputs to the system. Such a weighting sequence may be estimated by applying a known input sequence and correlating the input and output. It has been found<sup>2</sup> that a pseudo-random binary sequence (PRBS) is particularly suitable for this type of work. In the present study such a sequence was generated by digital computer and used to control the availability of food to an animal in a test apparatus. The availability of food is therefore regarded as the input to the system. The output of the system is a direct measure of the animal's water consumption, which was not constrained in any way, and was recorded on-line by the computer.

The computer was programmed to correlate the output with the input, and to calculate appropriate weighting sequences. The autocorrelation function of the PRBS input is given by the following equation

$$\begin{aligned} \phi_{uu}(sT) &= \frac{1}{N} \sum_{t=1}^N u_t \cdot u_{t-s} = a^2 \text{ where } s = 0 \\ &= -\frac{a^2}{N} \text{ where } s \neq 0 \end{aligned}$$

where  $a$  is the effective amplitude of the input, and  $N$  is the number of sequences. The input-output cross correlation function is given by the following equation

$$\phi_{yu}(sT) = \frac{1}{N} \sum_{t=1}^N y_t \cdot u_{t-s}$$

From these two functions it can be shown<sup>3</sup> that

$$W_s = \frac{1}{(N+1)a^2} \left\{ \sum_{t=1}^N y_t \cdot u_{t-s} + \text{an error term} \right\}$$

This function, which gives the impulse response, is calculated by the computer. In this context, the impulse response corresponds to the drinking function in response to a hypothetical pulse of food availability.

The experiment reported here involves a single Barbary dove (*Streptopelia risoria*), which had previously been used in a study of feeding and drinking *ad libitum*, and found to be similar to other individuals in this respect. The animal was housed, for the duration of the experiment, in a Skinner box installed inside a climatic cabinet maintained at 20° C, the same conditions as were used in the previous study. During

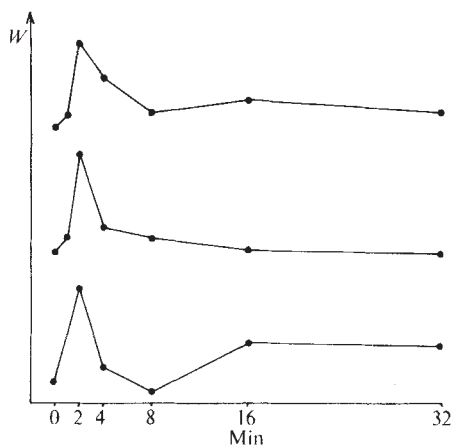


Fig. 1 Examples of impulse responses from three tests within the same week, in the undeprived animal.

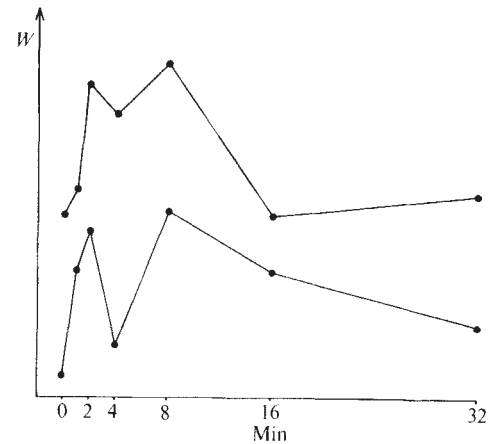


Fig. 2 Examples of impulse responses from two tests within the same week, in the animal deprived of food for one day prior to each test.

the 12-h artificial day, the subject was able to obtain water *ad libitum* by pecking at a green illuminated key to obtain 0.1 cc water per peck. Each peck was followed by a 10-s period during which the key illumination was turned off and the reward mechanism made inoperative (time out). When a red key was illuminated, the subject could obtain 3-s access to wheat per peck, followed by a 7-s time out. Thus the maximum possible reward rate was six rewards  $\text{min}^{-1}$  for both food and water. The illumination of the red key was controlled by computer in accordance with the PRBS, being turned on and off at random intervals. Pecks at the green key were recorded directly by the computer. The PRBS was arranged so that the minimum on/off time was 1 min, the maximum being 7 min. These experimental parameters were calculated so as to ensure that the subject was kept sufficiently hungry to peck at the red light whenever it was illuminated. The results showed that this invariably happened, although the animal did often break away from feeding and start drinking while the red light was still illuminated. PRBS tests were run for 12-h periods on alternate days. Between tests the animal was allowed to obtain food and water *ad libitum*, except on those days that it was deprived of food to determine the effect of hunger on the impulse response.

Examples of impulse responses calculated for the undeprived animal are illustrated in Fig. 1. These results indicate a strong tendency for drinking to occur about 2 min after food is made available. This finding is consistent with the view that normal drinking is highly dependent on feeding in doves<sup>4</sup> as in rats<sup>5,6</sup>. After food deprivation (Fig. 2), there is an additional tendency for drinking to occur about 10 min after feeding. This is probably due to systemic shifts in water balance, as food is released more rapidly from the crop after food deprivation<sup>7</sup>.

The conclusions reached on the basis of these results are extremely tentative, because the study is in its early stages. But a number of observations on the method may be appropriate. The PRBS method has a number of advantages, which make it particularly well suited to behavioural systems analysis. (a) It involves a binary input function, which can easily be produced, compared with a sinusoidal function, for example. The form "stimulus on—stimulus off" is more likely to be relevant to the behavioural situation than a continuously varying function. (b) Determination of the impulse response can be carried out in a single experimental session, whereas frequency analysis normally requires a separate session for each frequency used<sup>1</sup>. (c) The PRBS can be small in amplitude, thus inducing minimal disturbance during normal operation of the system. (d) The repetitive nature of the test signal makes the correlation function particularly immune to noise in the system. The main disadvantages are that non-linearities are difficult to spot, and



may lead to misinterpretation of the results. Secondly, a considerable amount of calculation is required, and access to a digital computer is necessary. It is not necessary, however, that computer facilities be available on-line, as the PRBS can be generated locally and the response recorded for subsequent analysis.

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## Suppression of Feeding by Intracranial Injections of Angiotensin

MANY species eat less than normal when deprived of water, and this may be due to a central nervous thirst factor inhibiting feeding<sup>1,2</sup>, thereby promoting water conservation<sup>3</sup>. It may be that many factors that increase thirst also reduce feeding, and this appears to be true for hypertonicity<sup>4</sup> and hyperthermia<sup>5</sup>. The recent finding<sup>6</sup> that drinking can be induced by intracranial injection of angiotensin in rats suggests that angiotensin may also suppress feeding.

A starving animal, which has just been allowed to start eating, stops eating and starts drinking when injected with angiotensin<sup>6</sup>. This effect could be due either to inhibition of eating by a thirst factor or to behavioural competition<sup>7</sup> in which angiotensin-induced thirst overrides hunger<sup>6</sup>. To demonstrate the former, it is necessary to test animals in a situation in which they have had prior experience of eating, but no experience of drinking.

Stainless-steel cannulae were implanted in the preoptic area of a number of rats, following a procedure described previously<sup>6</sup>. After the rats had recovered from the operation, each was tested in its home cage to ensure that drinking could be induced by intracranial injections of 1 µg angiotensin (Hypertensin, CIBA; 1 µg/µl.), administered in accordance with previously described procedures<sup>6</sup>.

Six rats were maintained on a 23-h food deprivation schedule, and were trained to press a bar for 45 mg food pellets in a Skinner box. Rewards were delivered on a random interval schedule, with a mean interval of 30 s (VI 30 s). After at least 7-h training sessions, each rat was given a 30-min session in the Skinner box with access to food only. Rewards obtained were recorded as a function of time, and each rat received experimental or control treatment after the first 5 min. After each session each rat was given 10-min access to water, but not to food, in its home cage. The amount of water consumed during this period was recorded. Details and results of the testing procedure are given in Table 1.

In every rat tested intracranial angiotensin depressed feeding behaviour in comparison with the control condition. During the last 15 min of the test, performance declines as a result of satiation, and these data are not reliable. Although more food was eaten by rats in the control tests, more water was drunk after tests in which the rats were injected with angiotensin 25 min previously. Feeding invariably recovered before the end of each session. It thus seems that a single injection of angio-

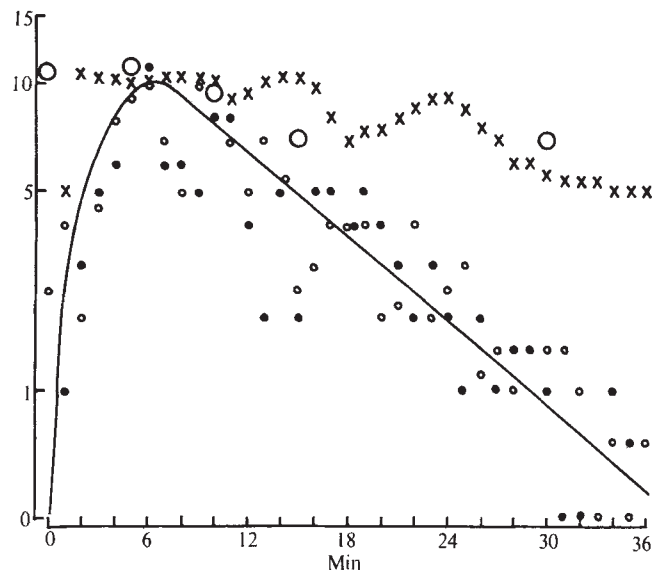


Fig. 1 Effect of 1 µg intracranial angiotensin on operant feeding and drinking. *a*, Depression of feeding in hungry rats following a single injection, showing data from two (open and closed small circles) of the rats tested (ordinate-control minus experimental score gives magnitude of depression in responses min<sup>-1</sup>). *b*, Depression of feeding following multiple, computer controlled, injections. Solid line indicates the calculated impulse response. *c*, Stimulation of drinking in non-deprived rats following a single injection. Crosses give averaged results of the three rats tested (ordinate-responses min<sup>-1</sup>). *d*, Amount consumed in drinking tests conducted at various intervals (abscissa) following a single injection (ordinate-ml. water consumed). Averaged results indicated by large circles.

tensin has a more prolonged effect on the drinking system than it does on the feeding system.

In order to assess more exactly the time-course of the suppression of feeding by angiotensin, the data from three rats were analysed in greater detail. The record of rewards obtained by rats injected with 1 µg angiotensin was subtracted, minute by minute, from that of the saline control, and this difference score was subjected to a running average with a time span of 3 min. The results obtained from the three rats were in substantial agreement, showing a peak suppression at about 6 min after injection, followed by an exponential return to normal by about 30 min after injection. Examples from two rats are given in Fig. 1. In order to compare the effects of angiotensin on feeding and drinking, three different rats were trained to obtain 0.1-ml. water rewards by working in a Skinner box (VI 30 s). These were tested in an undeprived condition but no food was available in the test cage. A single 1-µg intracranial injection of angiotensin was given and the averaged results are illustrated in Fig. 1. It seems that angiotensin acts more quickly, and has a more prolonged effect, on operant drinking than on feeding.

In order to assess the decay in effectiveness of angiotensin, a further seven rats were given a series of daily tests, in which water was withheld for a specified period following intracranial injection. The rats were undeprived, and were allowed access to water in a drinking column, either immediately after the

Table 1 Effect of Angiotensin Injections on Feeding

| Subjects     | Treatment        | Mean food rewards in first 15 min |        |        | Post-session drinking test |
|--------------|------------------|-----------------------------------|--------|--------|----------------------------|
|              |                  | 5 min                             | 10 min | 15 min |                            |
| Rats 1-3     | 1 µg angiotensin | 55.2                              | 39.3   | 31.6   | 12.2 ml.                   |
|              | No injection     | 62.3                              | 71.6   | 90.0   | 1.6 ml.                    |
| Rats 4-6     | 1 µg angiotensin | 48.1                              | 29.5   | 23.1   | 14.2 ml.                   |
|              | Isotonic saline  | 47.3                              | 55.0   | 59.0   | 4.0 ml.                    |
| Rats 1, 4, 5 | 2 µg angiotensin | 36.0                              | 17.0   | 10.4   | 10.4 ml.                   |
|              | Isotonic saline  | 37.5                              | 44.5   | 36.0   | 2.6 ml.                    |

injection or 5, 10, 15, 30 or 60 min after the injection. When each rat attained a satiation criterion of 5 min without further drinking, the amount of water consumed was measured. The amounts drunk by two rats tested with 1- $\mu$ g doses of angiotensin are indicated in Fig. 1. The decay rate of rats tested with other doses was essentially the same, and from these results it appears that the time course of VI operant drinking is concomitant with the decay of dipsogenic action of angiotensin.

To investigate further the inhibition of feeding by angiotensin an attempt was made to determine the impulse response<sup>8</sup> of the system by means of computer-controlled intracranial stimulation. A digital computer was used to turn a syringe-driver on and off, in accordance with a pseudo-random binary sequence (PRBS). When the driver was on, angiotensin was injected intracranially, at a constant rate (5–20  $\mu$ g h<sup>-1</sup>), calculated to provide an adequate dose over a period of 2 h. The effect on operant feeding behaviour was determined in the usual manner, except that the reward rate was reduced (VI, 2 min) to diminish the effects of satiation. A record of bar-presses was fed into the computer on-line, and the computer was programmed to calculate the impulse response relating intracranial injection to operant feeding behaviour, in accordance with previously described principles<sup>8</sup>. The impulse response gives the effect on the feeding system of a hypothetical impulse injection of angiotensin. The results proved to be unsatisfactory in certain cases because of the difficulty of relating dose level and hunger level. Meaningful results were obtained in a number of cases, however, and an example is illustrated in Fig. 1. In general, the results of the PRBS experiments show that the impulse responses calculated by computer are often similar to those obtained by measuring transient depression of feeding following a single large injection of angiotensin. Although the results are sometimes difficult to interpret, this method of assessing the behavioural effects of chemical stimulation of the brain seems promising.

Two main points emerge from these preliminary studies of the effect of intracranial injections of angiotensin on feeding behaviour. (a) Intracranial angiotensin stimulates drinking in the undeprived rat more quickly than it inhibits feeding in the hungry rat. (b) The effect on drinking is more prolonged than that on feeding.

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## Diffusion of Small Non-Electrolytes across Liposome Membranes

Lieb and Stein<sup>1</sup> proposed recently that biological membranes behave as a homogeneous polymer network (such as rubber) with respect to the diffusion of small molecular weight non-electrolytes. In the light of accumulating evidence that substantial areas of most cell or cell organelle membranes are composed of a bimolecular sheet of phospholipids or other lipids, we have tested the hypothesis using a model system with structure and composition which are simple and well defined.

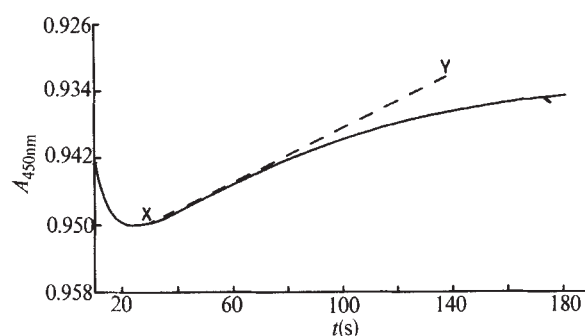


Fig. 1 Time course of the changes in absorbance after rapid addition of 0.18 ml. (8 mM lipid concentration) suspension of liposomes formed in 20 mM KCl into 2.5 ml. 100 mM ethylurea at 10° C. Liposomes were prepared from a mixture of egg yolk lecithin, cholesterol and phosphatidic acid in a molar ratio 48 : 48 : 4 respectively. Print-out readings of the absorbance changes were taken at least every second. From a sequence of readings taken after the minimum, a maximum slope was calculated. The portion of the straight line XY common to the absorbance curve illustrates the extent of the sequence that can be used for calculating the slope for ethylurea.

Smectic mesophases (liposomes)<sup>2</sup> exhibit changes in light scattering when osmotically driven water is moved into or out of the multimembraned structures<sup>3</sup>. With this technique, but using cholesterol-containing liposomes<sup>4</sup> mixed rapidly with hypertonic solutions of solutes at 10° C, the osmotic movements of water and solutes could be so slowed that two stages could be resolved (Fig. 1). The initial slope after mixing (complete within 500 ms) represents an increase in optical density and is indicative of a shrinking due to the high outside-inside solute gradient. This slope terminates at a minimum volume equilibrium point whose depth and time after mixing are related to the permeability coefficient of the external solute. Finally, there is a progressive decrease in the optical density representing an influx of external solute (down its concentration gradient) and its concomitant water. The time that the system takes to achieve the minimum volume<sup>5</sup> or the derivative of the rate of volume change at that minimum has been used by others<sup>6</sup> to determine the permeability coefficient of the permeant. We have taken the maximum gradient of the curve—just after the minimum—as a measure of our solute permeability. As this was the only parameter measured in our system, relative ratios—with respect to the value for urea—were calculated for the different solutes. Relative ratios of permeabilities determined by different methods have been found by others to be in good agreement in spite of the large amounts of bulk flow that can be involved in some of these determinations<sup>7</sup>.

Calculating the relative diffusion coefficients from our measured permeability values (equation 4 of ref. 1) for some twenty non-electrolytes and plotting these logarithmically against their molecular weights, we obtain a distribution as shown in Fig. 2. We have taken as values for  $K$  in liposomes the corresponding olive oil/water partition coefficients<sup>8</sup>, on the basis that the average chain composition of the egg yolk lecithin used is about eighteen carbon atoms. A regression line for these points has a negative slope of 0.017 with a square root of the residual variance ( $\sigma$ ) of 0.21 and a correlation coefficient ( $r$ ) of 0.93. The corresponding plot of these diffusion values against the logarithm of the molecular weight of the solutes (relative to methanol) has a negative slope of  $3.6 \pm 0.4$ ,  $\sigma = 0.24$  and  $r = 0.91$ . These values are rather similar to those computed by Stein and Nir for Chara and for synthetic polymer membranes<sup>9</sup>. The diffusion coefficient for trimethyl citrate, it will be seen in Fig. 2, is significantly too high, but so it was for Chara. No explanation for this deviation is offered.

When the measured permeabilities for the alkyl-substituted ureas and aliphatic amides are plotted against their olive oil/water partition coefficients an interesting and significant



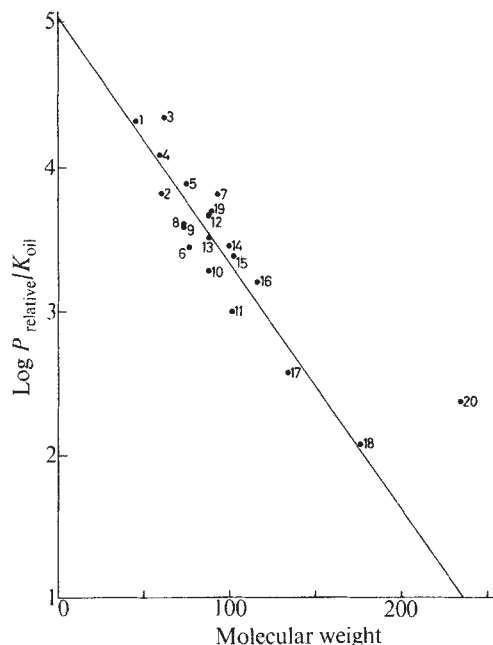


Fig. 2 Diffusion of non-electrolytes within liposomes estimated as the ratio of the measured permeability values to their olive oil/water partition coefficient. Ordinate:  $P_{\text{relative}}/K_{\text{oil}}$  ( $P_{\text{urea}} = 1$ ). Abscissa: molecular weight of the permeant. The linear regression line was calculated by the method of least squares. The negative of the slope of this line is 0.017. Permeants have been assigned the following numbers: 1, formamide; 2, urea; 3, ethylene glycol; 4, acetamide; 5, methylurea; 6, 1,2-propanediol; 7, glycerol; 8, dimethyl formamide; 9, propionamide; 10, butyramide; 11, valeramide; 12, N-N-dimethylurea; 13, ethylurea; 14, succinimide; 15, malonamide; 16, N-N-diethylurea; 17, monacetin; 18, diacetin; 19, lactamide; 20, trimethylcitrate. The regression line was calculated with the exclusion of trimethylcitrate.

selectivity is exhibited by the model system in the conditions used (Fig. 3). It can be seen that the increase in the permeability in both series tends to become smaller with each additional  $\text{CH}_3$  group (with the exception of formamide) and that the corresponding members of the amide series are more permeable than the ureas at equal olive oil/water partition coefficient.

The barriers to transfer of non-electrolytes appear to have the same fundamental structure in smectic mesophases, hydrophobic polymer networks and biological membranes.

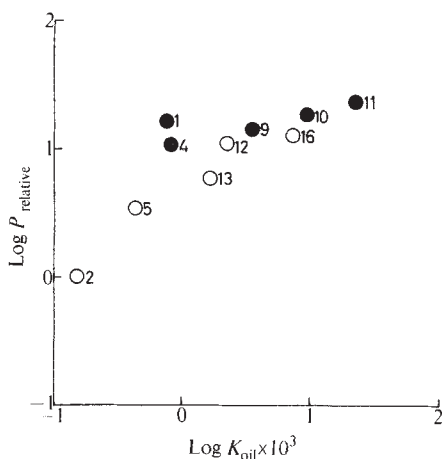


Fig. 3 Relative permeabilities of liposomes to non-electrolytes of different oil solubility and molecular weight. Ordinate:  $P_{\text{relative}}$  ( $P_{\text{urea}} = 1$ ). Abscissa: olive oil/water partition coefficient. ●, Alkyl-substituted ureas; ○, aliphatic amides. Numbering of solutes as in Fig. 2.

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## Isolation of Infectious Pancreatic Necrosis Virus from White Suckers (*Catostomus commersoni*)

INFECTIOUS pancreatic necrosis (IPN) is an acute, highly contagious viral disease of salmonid fishes. The disease is characterized by explosive outbreaks of mortalities in salmonid fry. Epizootics in hatchery fishes are dreaded, and have characterized it as a major disease of hatchery salmonids. Although the occurrence of the disease has been well documented in hatchery salmonids, little is known of its occurrence and consequences in native fishes. We conducted a virus survey to determine the possible presence of IPN virus in fishes in the tailwaters of two IPN-infected trout hatcheries.

Various species of fishes were collected, by means of a portable a.c.-d.c. fish shocker<sup>1</sup>, from the tailwaters of two trout hatcheries known to contain IPN-infected brook trout (*Salvelinus fontinalis*). The specimens collected were placed on ice in the field, transported to the laboratory, and frozen at  $-20^\circ\text{C}$  until assayed. Table 1 shows the details of the fishes collected and examined.

In the virus assays of the brook trout, portions of the spleen and kidney of individual fish were examined employing the method outlined by Hoffman and co-workers<sup>2</sup>. Visceral homogenates of the other fish species were examined for virus following the same method. The rainbow trout gonad (RTG-2) cell line established by Wolf and Quimby<sup>3</sup> was used in all the virus assays.

Only one isolation of virus was made from all the fishes examined. This came from one visceral homogenate prepared from ten yearling white suckers (*Catostomus commersoni*). These suckers were taken from the tailwaters of hatchery B, directly below a pond holding 196,000 yearling brook trout known to have 34% incidence of infection with IPN. The virus isolate was neutralized by IPN-antisera, and electron microscopic examinations of thin sections of RTG-2 cell cultures infected with it revealed reovirus-like particles consist-

Table 1 Fishes examined from Tailwaters of IPN-Infected Hatcheries

| Common name   | Species<br>Scientific name   | No. examined |            |
|---------------|------------------------------|--------------|------------|
|               |                              | Hatchery A   | Hatchery B |
| White sucker  | <i>Catostomus commersoni</i> | 0            | 485        |
| Yellow perch  | <i>Perca flavescens</i>      | 0            | 8          |
| Northern pike | <i>Esox lucius</i>           | 0            | 15         |
| Brook trout   | <i>Salvelinus fontinalis</i> | 20           | 2          |
| Shiners       | <i>Notropis spp.</i>         | 0            | 51         |
| Totals        |                              | 20           | 561        |

tent with previous findings in this laboratory and those of other workers<sup>4,5</sup> for IPN virus.

This is the first report of the isolation of IPN virus from non-salmonid fishes. Although the visceral homogenate from the suckers possessed an IPN titre of  $10^{3.1}$  TCID<sub>50</sub>, it does not necessarily prove these fish were actively infected with IPN. The IPN isolation may have been the detection of virus which had been ingested in infected food. Nevertheless, the fact that these fish did harbour IPN virus, together with Wolf's report<sup>6</sup> of IPN replicating in cell lines and explant cultures from a wide variety of fishes, suggests that white suckers and perhaps other non-salmonid fishes may play a role in the ecology and epizootiology of IPN.

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## Micro-electrode Measurements of the Transmembrane Potential of Chloroplasts and its Photoinduced Changes

MEMBRANE potential changes across the outer cytoplasmic membranes of green plant cells are associated with photosynthesis<sup>1</sup>. The question of the existence of electrical potential on chloroplast membranes is important for elucidating the connexion between electrogenesis and photosynthesis: light-induced generation of a potential difference on the chloroplast membrane is an essential feature of the chemiosmotic hypothesis<sup>2</sup>.

Mesophyll cells of *Peperomia metallica* usually have three to five giant chloroplasts with a diameter of 15–25  $\mu\text{m}$ <sup>3</sup>. With the use of new micro-electrode techniques<sup>4,5</sup>, membrane potentials were measured on bound and isolated chloroplasts, as well as those within the cell.

The medium for leaf sections was prepared according to White<sup>6</sup>. The medium for isolated chloroplasts contained 250 mg 'Ficoll' 10 ml.<sup>-1</sup> medium, 1 mg serum albumin 10 ml.<sup>-1</sup> medium; 0.01 M Tris-HCl buffer (pH 7.8) and 0.25 M sucrose<sup>7</sup>.

Electrode insertions were done in a small glass chamber, mounted on the stage of the microscope. Solution medium flowed continuously through the chamber. The glass micro-electrodes with tip diameters of less than 0.5  $\mu\text{m}$  were filled with 2.5 M KCl and had resistances about 40–50 Mohm. Micro-electrodes were inserted horizontally using an improved Zeiss micromanipulator. A hydraulic device with a step-wise motion (2  $\mu\text{m}$  per impulse in this case) was used for impaling chloroplasts. The other micro-electrode was inserted into the cytoplasm of the cell (Fig. 1). The recording and reference electrodes were connected to saturated calomel half-cells and potential changes measured on electrometer (input resistance

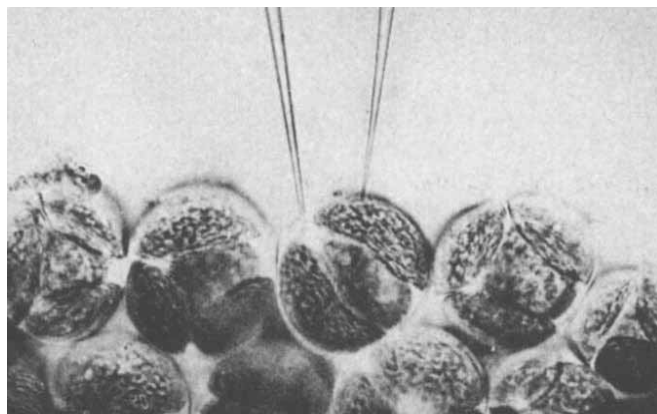


Fig. 1 Measurement of the chloroplast membrane potential in the leaf of *Peperomia metallica*, showing the measuring micro-electrode inserted in the chloroplast and the reference micro-electrode positioned within the cell.

10<sup>11</sup> ohm) coupled to a potentiometer and an oscilloscope. Oscilloscope traces were photographed.

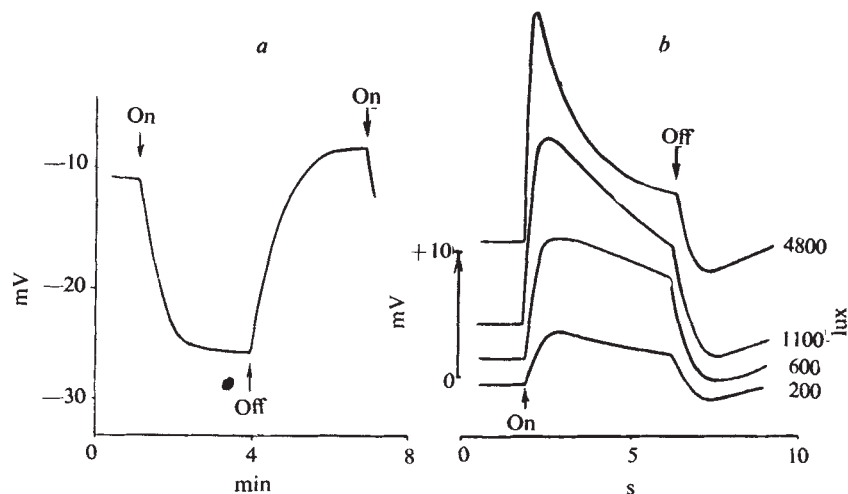
Chloroplasts were isolated using a microtechnique for producing extracts of mesophyll cells<sup>7</sup>. The upper epidermis of the leaf was stripped off, a few drops of extracting medium were placed on the exposed cells and the mesophyll region was cut about twenty times with a razor blade. The cell-free extract was then collected by a capillary pipette and transferred into the chamber. The chloroplast was drawn on to the fire-polished tip of a glass pipette (tip diameter about 15  $\mu\text{m}$ ) by suction from a syringe. The object was illuminated from underneath by heat-filtered light provided by a microscope incandescent lamp at various light intensities. The temperature of the medium in the chamber was measured by thermocouple with an accuracy of 1°–2° C. Most of the experiments were done at room temperature (17°–19° C). There was no detectable response to illumination on the part of the micro-electrode tip itself when it was outside the chloroplast. No light-induced potential could be produced by measuring the damaged chloroplast or after plugging the tips of the micro-electrodes with fragments from chloroplasts. Thus micro-electrodes show no detectable photovoltaic effects.

Potential differences between chloroplasts and cytoplasm measured *in situ* in different cells have either a positive value up to +15 mV, or a negative value up to 60 mV relative to the cytoplasm, and depend on the age of the leaf, the conditions, the position of the cell within the leaf blade and other factors. Illumination with "white" light (tungsten lamp) and red light (> 600 nm) induced both slow and fast changes in the membrane potential of chloroplasts; both could sometimes be seen in experiments with one chloroplast. Slow photoinduced potential changes are associated with a shift towards the negative by 10–20 mV during 1–3 min (light intensity of 4,000–5,000 lux), with a subsequent settling to a constant level. When the light is switched off, the potential returns to its initial value (Fig. 2a). On fast potential changes, there is a shift to the positive relative to the cytoplasm (by 10–30 mV during 0.01–0.05 s at a light intensity of 4,000–5,000 lux), with a subsequent settling to a constant level. The rising time of these potential changes is possibly less than 0.01 s, which is beyond the resolution of our instruments. When the light is switched off the potential returns to the initial level (Fig. 2b). Fast changes could be measured about 5–10 min after micro-electrode penetration, when they were completely reversible; if recording was up to 15 min, an increase in rising time and decay of the response was observed.

In a number of experiments both fast and slow photoinduced changes could be observed in the same chloroplast. In this case illumination produces a rapid photoelectric response followed by slower changes of the chloroplast potential of opposite sign. Fast photoinduced changes appear repeatedly under intermittent illumination, while slow photoinduced



Fig. 2 Two types of photoinduced changes of the chloroplast potential. Slow (light-intensity) 5,000 lux); fast (at different light intensities).



changes disappear after two or three consecutive periods of light and darkness.

The magnitude of fast photoinduced changes depends on the incident light intensity. At low intensities magnitude is proportional to the intensity of light, while at higher intensities (3,000–4,000 lux) saturation is observed. Changes in the temperature of the medium in the range 3°–17° C have practically no effect on the magnitude of fast photoinduced changes. The temperature coefficient  $Q_{10}$  within the temperature interval 5°–15° C is equal to 1–1.2. In the presence of  $5 \times 10^{-6}$  M 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) both slow and fast changes are inhibited, but they can be restored by the addition of  $10^{-4}$  M phenazine methasulphate, which also substantially increases (up to three times) the magnitude of fast photoinduced changes.

Action spectra for fast chloroplast potential changes were measured using interference filters, half-bandwidth 12 nm, for fourteen wavelengths in the range 410–725 nm. In all cases the relationship between light intensity and the magnitude of the changes was linear over the range used for the action spectrum determinations. The shape of action spectra (Fig. 3) with maxima of 420, 500, 680 nm corresponds to the absorption spectrum of intact leaves and is similar to the action spectrum of photosynthesis.

Measurements of the transmembrane potential of single isolated chloroplasts were also made. The potential difference between the chloroplast interior and outer medium was found to be from –20 to +15 mV.

Illumination of single isolated chloroplasts evoked a fast photoelectric response. The magnitudes of fast light-induced changes measured in isolated chloroplasts often exceeded those

measured in the cell and reached 50–75 mV. Light-intensity curves for the fast changes were identical to those described above. The addition of an uncoupler of phosphorylation (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP)  $10^{-5}$  M) to the extraction medium inhibits the magnitude of the fast changes by 70–80%.

The complete reversibility and great magnitude of the effect (up to 75 mV), the mode of action of inhibitors, and the shape of action spectra indicate that the photoelectric responses are not due to an artefact.

The occurrence of photoinduced changes differing in direction and rapidity seems to be determined by a different localization of the micro-electrode tip within the chloroplast (stroma, lamellar system), a different degree of injury of the chloroplast. It seems possible that rapid photoinduced changes are confined to the inner membranes and slow changes to the outer membrane.

The results of these experiments, in particular the variation of the sign of the transmembrane potential in darkness, and the presence of two types of photoelectric responses with opposite signs point to the complexity of the observed phenomena and indicate the presence of photoelectric responses in the membrane structures of chloroplasts which may be essential for the course of photosynthesis and functionally connected with the storage of energy during photosynthesis. In accordance with the conception developed by Mitchell<sup>2</sup> it may be assumed that the initial phase of the fast photoelectric response is determined by the separation of charges due to the photosynthetic transport of electrons and the transfer of  $H^+$ , while the following phase is determined by compensatory processes of ion transport through the membrane of thylacoids. In this context it is interesting that the rapid photoinduced changes described above correspond in time to the rapid photoinduced changes in the concentration of  $H^+$ , which were discovered by several authors in the chromatophores of bacteria and the chloroplasts of higher plants<sup>8,9</sup>. The observation of an inhibitory effect of the uncoupler (FCCP  $10^{-5}$  M) on the photoelectric response is consistent with chemiosmotic hypothesis.

Slow photoinduced response of the chloroplast potential may be determined by changes in the activity of cations in chloroplast and cytoplasm due to light induced  $H^+$  and  $K^+$  translocation.

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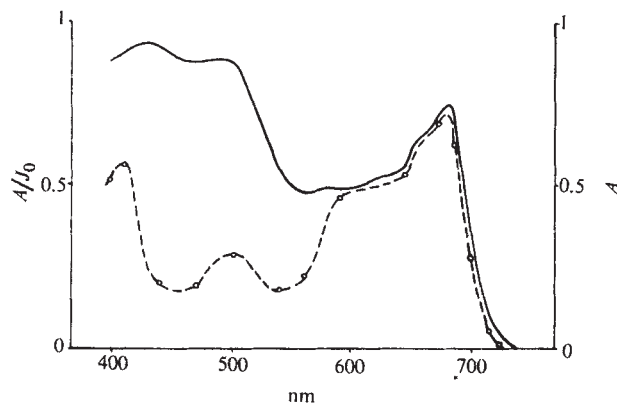


Fig. 3 Absorption spectrum of intact leaves of *Peperomia metallica* (—) and an action spectrum (---) of fast photoinduced changes of chloroplast potential ( $A/J_0$ —the amplitude of the effect related to the intensity of incident light ( $J_0$ ), in relative units).

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## Hydroxylations of Carbaryl by Soil Fungi

CARBARYL (1-naphthyl N-methylcarbamate) is an important pesticide with a half-life in soil of approximately 1 week. However, the possible formation of detoxication products by microorganisms that persist longer in soil has not been investigated.

There are many indications that carbaryl is hydrolysed chemically to 1-naphthol in various conditions<sup>1,2</sup>, and biological hydrolysis probably also occurs due to soil microbes<sup>3</sup>. The fungus *Gliocladium roseum* transforms carbaryl by a non-hydrolytic mechanism, whereby the insecticide molecule was hydroxylated at the methyl group forming 1-naphthyl N-hydroxymethylcarbamate, or on the aromatic ring forming 4- and 5-hydroxy-1-naphthyl methylcarbamate<sup>4</sup>. By using radiolabelled carbaryl, a further breakdown of the formed metabolites was indicated by a decrease of the total radioactivity in the hydrolytic as well as the hydroxylation products. However, the mechanism of the subsequent decomposition remains unclear.

We now describe the capacity of several common soil fungi to hydroxylate carbaryl. Most fungi were isolated from carbaryl-treated soil by the dilution plating method. *Aspergillus flavus*, *Mucor racemosus*, *Penicillium roqueforti* and *Fusarium roseum* came from the culture collection of the Department of Microbiology at Pennsylvania State University. All fungi were cultured on a rotary shaker at 28° C in the following nutrient medium: 0.01 mCi of methyl-<sup>14</sup>C-labelled carbaryl (specific activity, 26.4 mCi/mmol); carbaryl, 0.1 g; nutrient broth (Difco), 8.0 g; dextrose, 10.0 g; yeast extract (Difco), 1 g; NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, 6.0 g; and Na<sub>2</sub>HPO<sub>4</sub>, 0.5 g in 1.0 l. distilled water. The final pH was 6.0.

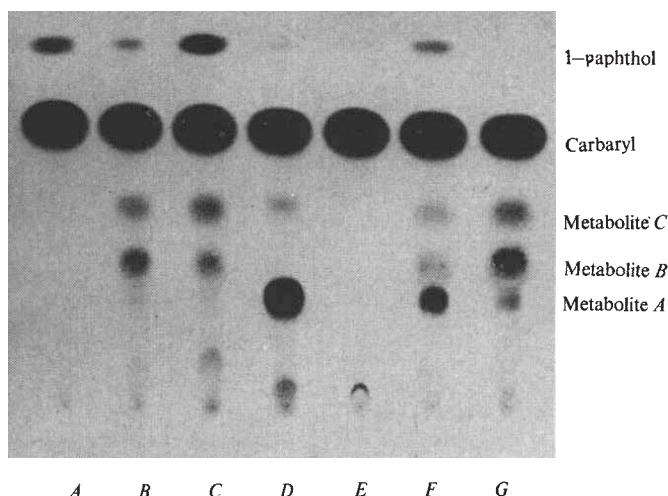


Fig. 1 Metabolic products of carbaryl after incubation with different soil fungi. Thin-layer plate was developed with an ether-hexane (4 : 1, v/v) mixture. A, Control medium; B, *Rhizopus* sp.; C, *Mucor* sp.; D, *Aspergillus terreus*; E, *Fusarium oxysporum*; F, *Gliocladium roseum*; G, *Penicillium* sp.—isolate 2.

The transformation of carbaryl was followed by thin-layer chromatography and identification of the radiolabelled spots. For this purpose, the culture filtrates of the different fungi were extracted with ether and aliquot samples of the concentrated ether extracts were applied on thin-layer plates<sup>4</sup>. The quantity of each radioactive metabolite was determined by scraping the specific *R<sub>F</sub>* area into a scintillation solution containing 4% 'Cab-O-Sil' (thixotropic suspension powder) and then counted with a liquid scintillation spectrometer.

After hydrolysis with a 15% potassium hydroxide solution and spraying with 0.1% methanolic *p*-nitrobenzene diazonium fluoroborate<sup>5</sup>, metabolites A, B and C gave a yellow, pink and violet colour, respectively. Without hydrolysis, metabolite A did not react whereas metabolites B and C formed a pink and yellow spot, respectively. A chromatogram demonstrating representative patterns indicating the formation of products from carbaryl by various fungi is shown in Fig. 1. Metabolite A was identified as 1-naphthyl N-hydroxymethylcarbamate, metabolite B as 4-hydroxy-1-naphthyl methylcarbamate and metabolite C as 5-hydroxy-1-naphthyl methylcarbamate by ultra-violet, infrared and mass spectroscopy in the culture solution of *Gliocladium roseum*<sup>4</sup>. In further spectroscopic identification

Table 1 Formation of Hydroxylated Metabolites from Methyl-<sup>14</sup>C-Labelled Carbaryl by Various Soil Fungi

| Fungus                                   | <i>R<sub>F</sub></i> range<br>0.20–0.30<br>(1-naphthyl N-hydroxy-<br>methylcarbamate) | <i>R<sub>F</sub></i> range<br>0.32–0.39<br>(4-hydroxy-1-naphthyl<br>methylcarbamate) | <i>R<sub>F</sub></i> range<br>0.44–0.51<br>(5-hydroxy-1-naphthyl<br>methylcarbamate) | <i>R<sub>F</sub></i> range<br>0.60–0.72<br>(Carbaryl) |
|------------------------------------------|---------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|-------------------------------------------------------|
| <i>Aspergillus flavus</i> Link ex Fries  | 4,342                                                                                 | 126                                                                                  | 228                                                                                  | 11,723                                                |
| <i>Aspergillus fumigatus</i> Fresenius   | 0                                                                                     | 0                                                                                    | 0                                                                                    | 19,159                                                |
| <i>Aspergillus niger</i> Van Tieghem     | 739                                                                                   | 140                                                                                  | 305                                                                                  | 16,000                                                |
| <i>Aspergillus terreus</i> Thom.         | 4,854                                                                                 | 59                                                                                   | 262                                                                                  | 12,391                                                |
| <i>Aspergillus</i> sp.                   | 2,257                                                                                 | 186                                                                                  | 334                                                                                  | 11,649                                                |
| <i>Fusarium oxysporum</i> Schlechtendahl | 0                                                                                     | 0                                                                                    | 0                                                                                    | 19,722                                                |
| <i>Fusarium roseum</i> Link              | 569                                                                                   | 0                                                                                    | 0                                                                                    | 16,154                                                |
| <i>Fusarium</i> sp.                      | 0                                                                                     | 0                                                                                    | 0                                                                                    | 20,882                                                |
| <i>Geotrichum candidum</i> Link          | 0                                                                                     | 0                                                                                    | 0                                                                                    | 19,069                                                |
| <i>Gliocladium roseum</i> (Link) Thom.   | 829                                                                                   | 254                                                                                  | 408                                                                                  | 15,443                                                |
| <i>Helminthosporium</i> sp.              | 63                                                                                    | 235                                                                                  | 82                                                                                   | 16,045                                                |
| <i>Mucor racemosus</i> Fresenius         | 134                                                                                   | 1,275                                                                                | 834                                                                                  | 14,503                                                |
| <i>Mucor</i> sp.                         | 65                                                                                    | 406                                                                                  | 666                                                                                  | 14,228                                                |
| <i>Penicillium roqueforti</i> Thom.      | 0                                                                                     | 0                                                                                    | 0                                                                                    | 17,406                                                |
| <i>Penicillium</i> sp.—isolate 1         | 291                                                                                   | 0                                                                                    | 0                                                                                    | 16,719                                                |
| <i>Penicillium</i> sp.—isolate 2         | 266                                                                                   | 3,342                                                                                | 1,003                                                                                | 13,495                                                |
| <i>Rhizopus</i> sp.                      | 122                                                                                   | 1,400                                                                                | 719                                                                                  | 14,115                                                |
| <i>Trichoderma viride</i> Per. ex Fries  | 102                                                                                   | 117                                                                                  | 95                                                                                   | 15,942                                                |
| Control medium                           | 0                                                                                     | 0                                                                                    | 0                                                                                    | 18,769                                                |

Amounts of radioactivity (d.p.m.) detected after thin-layer analyses of the ether extract from 5 day old growth media.



with some of the detectable metabolites, the ultraviolet and infrared spectra of metabolite *A* from *A. terreus* were identical to authentic 1-naphthyl N-hydroxymethylcarbamate. The ultraviolet and infrared spectra of metabolites *B* and *C* from *Mucor* sp. and *Penicillium* sp. corresponded to authentic 4-hydroxy- and 5-hydroxy-1-naphthyl methylcarbamate, respectively. The spot appearing in the  $R_F$  area of 0.86–0.90 (Fig. 1) co-chromatographs with 1-naphthol. The uninoculated control medium shows the formation of 1-naphthol after 5 days of incubation which implies chemical decomposition of carbaryl. The varying disappearance of 1-naphthol in the fungal cultures indicates a different capability of the fungi to metabolize the chemical degradation product.

Most of the investigated soil fungi could metabolize carbaryl to hydroxylated derivatives (Table 1); however, the products varied qualitatively as well as quantitatively with the various fungi. All the *Aspergillus* species, except *A. fumigatus*, hydroxylated carbaryl. 1-Naphthyl N-hydroxymethylcarbamate was found to be the major metabolic product; approximately 28% of the radiocarbon from the applied  $^{14}\text{C}$ -labelled insecticide was detected in this metabolite with *A. terreus*. In contrast to the dominance of this product, isolates of *Penicillium* sp. (isolate 2), *Mucor* sp. and *Rhizopus* sp. had a stronger tendency to hydroxylate carbaryl in the ring position, whereas a weak ability for the formation of the side-chain hydroxylated metabolite was observed. With all the cultures, it seems that a decrease of radioactivity from carbaryl was correlated with the amount of metabolites formed.

Thus it can be concluded that soil fungi alter the insecticide carbaryl by side-chain and ring hydroxylations. The same hydroxylated metabolites have also been found as plant, insect and animal biotransformation products<sup>6,7</sup>. Since this detoxication mechanism seems to be widespread among fungi, it will be necessary to clarify the fate of the formed products in soil and other ecosystems.

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## Response of the Alga *Chlorella sorokiniana* to $^{60}\text{Co}$ Gamma Radiation

THE unicellular green alga *Chlorella sorokiniana*, a thermophilic (39° C) mutant of *Chlorella pyrenoidosa*, Emerson strain, is often considered for the bioregeneration of oxygen from waste carbon dioxide in closed ecological life support systems operated in radiation environments. The response (survival) of this alga to ionizing radiation is not known. The experimental results presented here are the first such data obtained for this species.

Stock cultures of *C. sorokiniana* were grown autotrophically in 100 ml. of sterile, modified Knop's solution<sup>1</sup> contained in

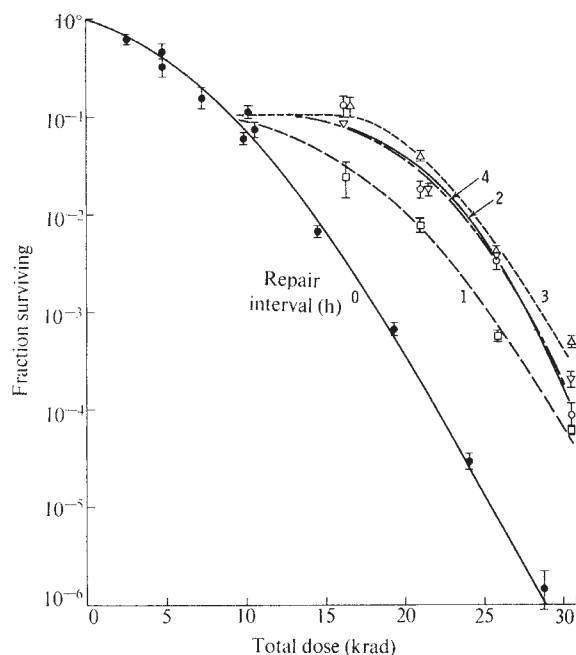


Fig. 1 Experimental survival curves after  $^{60}\text{Co}$  gamma irradiation. Survival of cells of age 1 h after single dose (no period for repair) is given by  $\bullet$ . Cells surviving a first dose of 9.6 krad (at age 1 h), followed by a time interval for repair of sublethal damage and then second doses of 5, 10, 15 and 20 krad, are given by  $\square$  (1 h repair),  $\circ$  (2 h repair),  $\triangle$  (3 h repair), and  $\nabla$  (4 h repair).

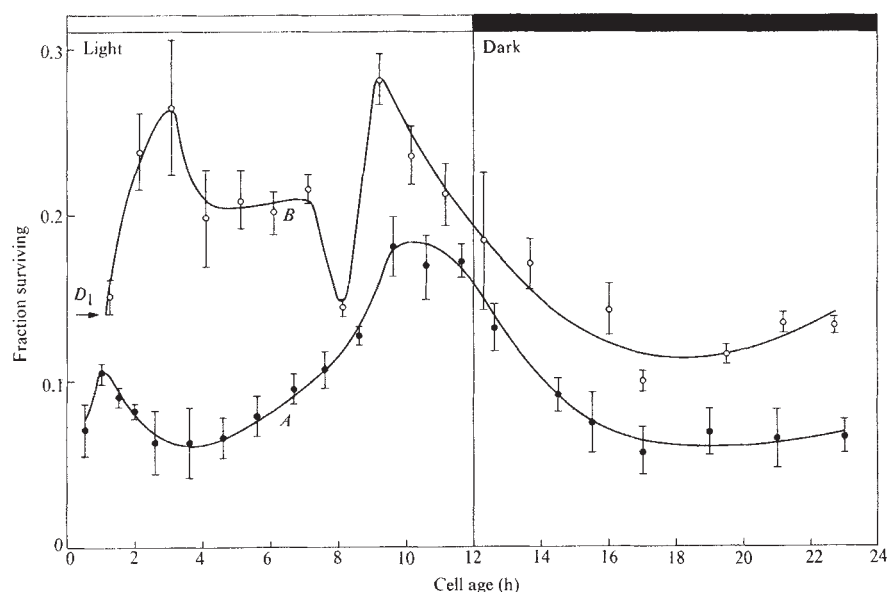
250 ml. glass flasks in an atmosphere of 5%  $\text{CO}_2$  in air, at a temperature of 37.5° C, in a gyratory incubator-shaker. Illumination was provided by an overhead bank of cool-white, high-output fluorescent lamps (5,400 lumens  $\text{m}^{-2}$ ) operated in a cyclic illumination sequence of 12 h light and 12 h dark to induce division synchrony. The beginning of each light period was considered to be zero hour with respect to cell age.

The response of the algal cell to  $^{60}\text{Co}$  gamma radiation was measured in terms of survival (colony-forming ability). Aliquots of the synchronized stock culture were exposed at an absorbed dose rate of 15 krad  $\text{min}^{-1}$  in fully aerobic conditions. Ferrous sulphate dosimetry was performed with an identical geometry. Irradiated samples and unirradiated controls were diluted, as required, and plated on modified Knop's agar (1.5%) supplemented with 0.01 M glucose. The plates were incubated in air at an ambient temperature of 30° C under incandescent light (2,700 lumens  $\text{m}^{-2}$ ) and scored 6 days later for visible colonies.

The dependence of survival on absorbed dose was determined for a 1 h old synchronous culture by exposing aliquots to different single doses and measuring the surviving fraction. Fig. 1 shows this survival curve (0 h repair interval). The dose  $\bar{D}_0$  necessary to reduce the surviving fraction in the exponential region by  $e^{-1}$  was found to be 1.7 krad. For comparison, the  $\bar{D}_0$  value for an asynchronous culture is 3.1 krad (unpublished data).

By administering the doses in two fractions separated by known periods of time, sublethal damage repair was demonstrated and the repair rate was estimated. A first dose ( $D_1$ ) of 9.6 krad was administered to 1 h old cells. After varying periods of time to allow for repair (1, 2, 3 or 4 h), graded second doses (5, 10, 15 and 20 krad) were given and the surviving fraction was measured. The two-dose survival curves in Fig. 1 show that with increasing periods of time between doses, the shoulder of the survival curve reappears. This is interpreted as evidence for the repair of sublethal damage induced by the first dose<sup>2,3</sup>. The survival curve with 1 h of repair falls between the one-dose curve and the two-dose

**Fig. 2** Survival as a function of cell age after  $^{60}\text{Co}$  gamma irradiation. Curve *A* (●) shows the fraction of cells surviving a single dose of 10 krad given at various cell ages. Curve *B* (○) shows the fraction of cells surviving an equivalent total doses split into two fractions:  $D_1 = 5.7$  krad administered to cells of 1 h,  $D_2 = 4.3$  krad given subsequently at the cell ages indicated. The time between doses allows for the repair of sublethal damage.



curve with 2 h of repair. The survival curves for repair intervals of 2, 3 and 4 h essentially coincide. Maximal repair therefore is achieved within 3 h. At large doses, repair increases survival by two orders of magnitude over that for irradiations with an equal dose without repair.

The variation of radiosensitivity with cell age (the age-response function) was studied by subjecting aliquots of the synchronized stock culture to a single dose of 10 krad at various cell ages during the 24 h life cycle (imposed by the illumination conditions) and measuring the surviving fraction. The survival (radiation resistance) of *C. sorokiniana* has a pronounced age dependence (Fig. 2, curve *A*). The first conspicuous survival peak occurs at age 1 h. It seems to correlate with DNA synthesis in the chloroplast<sup>4</sup>. The low value of survival at age 3–5 h coincides with the peak of photosynthetic activity in the age interval of 3–4 h<sup>5</sup>. My observation also correlates well with the finding of Davies<sup>6</sup> that, in the alga *Chlamydomonas reinhardtii*, the radiation sensitivity increases between the ages of 3 and 5 h. This suggests that photosynthesis or its products (in particular, a high intracellular oxygen concentration) may be a cause for the survival minimum at about 3 h. The subsequent increase in survival to a maximum value near age 10 h may result from the increase in the RNA content of the cell during this portion of the cell cycle. Other investigators have found RNA synthesis to be necessary for repair of radiation damage<sup>7,8</sup>. Further, the latter part of the DNA synthesis phase is often observed (in other unicellular organisms and cells cultured *in vitro*) to be refractory with respect to radiation-induced reproductive death and may contribute to the observed survival maximum at 10 h. Čecháček and Hillová<sup>9</sup> found a 35% increase in  $\bar{D}_0$  values during DNA synthesis in *Chlamydomonas*. The low survival level during the last eight hours of the dark cycle and at the beginning of the light cycle may be dictated by a relatively low level of RNA synthesis in the nascent cells and/or some radiosensitive step(s) in the synthesis of chlorophyll precursors that may begin in the daughter cells shortly after liberation in the dark period<sup>10</sup>.

The influence of sublethal damage repair on the age-response function was studied by exposing synchronized samples to a 10-krad total dose split into a first dose (5.7 krad) given at age 1 h and a second dose (4.3 krad) given after repair intervals of varying duration, ranging over the entire cell cycle. Survival with repair is always higher than that without repair, regardless of the cell's age (Fig. 2, curve *B*). The first survival peak occurs at age 3 h, 2 h after the administration of the first dose. This time interval is comparable with that required for maximal repair of sublethal damage (Fig. 1). Survival decreases slightly thereafter and remains constant for 3 h. This three-hour

period may reflect a delay in cell progression resulting from the blockage of photosynthesis that provides the necessary precursors for macromolecular synthesis<sup>11</sup>. The difference in survival between the 8 h minimum and the 9 h maximum for the two-dose curve (*B*) is about the same as that between 3.5 and 10 h for the single dose curve (*A*), suggesting that by 10 h, for both the single and two-dose situation, the same part of the cell cycle has been traversed. Relative changes in cell radiosensitivity thereafter appear to follow those expected from normal cell cycle kinetics (Fig. 2, curve *A*).

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## Amphipithecus Revisited

IN an article by Simons<sup>1</sup> on the relationships of *Oligopithecus* and *Amphipithecus*, the new figures of *Oligopithecus* and accompanying inferences show unequivocally that this Fayum primate is a catarrhine. My doubts<sup>2</sup> concerning the phyletic affinities of *Oligopithecus*, expressed while discussing the relationships of *Amphipithecus*, have been dispelled; it is good to see photographic illustrations published of this unique specimen of an early catarrhine.



In addition to the evidence presented for *Oligopithecus*, Simons argues that *Amphipithecus* is a catarrhine, and he disputes my suggestion that it is probably lemuriform. I shall not deal with his arguments why *Amphipithecus* is not a condylarth, it is obviously not, but it should be mentioned that, contrary to his statement that condylarths are absent from Asia, both arctocyonid and mesonychid condylarths occur in the Eocene of Mongolia.

Simons apparently did not consider my analysis of the crown pattern of *Amphipithecus*. He has recently also dismissed<sup>3</sup> the conclusions of Van Valen and Sloan<sup>4</sup> regarding the genus *Purgatorius*. These authors based their inferences also on teeth (the only evidence available so far). Simons doubted the primate affinities of this genus and questioned the validity of the hypodigm of *Purgatorius unio*. The sixteen well preserved teeth which make up the hypodigm of this taxon have, however, been correctly allocated and astutely analysed by their describers, and their primate affinities have been confirmed by several students of early Tertiary mammals who are familiar with the relevant faunas and groups of mammals. Dismissing the analysis of molar patterns in various fossil primates is no substitute for knowledgeable criticism.

I have little to add to my analysis and comparisons of the teeth in *Amphipithecus*. The wear on the molars of *Oligopithecus* only confirms my postulated ancestral catarrhine talonid pattern. As Simons's illustrations show<sup>1</sup>, *Oligopithecus* as well as the other Fayum catarrhines share the distinct lingually placed and somewhat prominent hypoconulid—Eocene lemuroids and *Amphipithecus* lack this type of talonid construction. Simons stated, after dismissing the dental evidence, that Colbert<sup>5,6</sup> has proved the dental affinities of the Burmese primate to be with catarrhines. But perusal of Colbert's pertinent papers fails to show this—Colbert neither did nor claimed to have analysed the dental evidence from the details of the crown patterns. His figures, drawings of *Amphipithecus*, are inaccurate. Simons's Fig. 1 is not from Colbert 1935, but they are photographs taken by Chester Tarka at the American Museum of Natural History. *Amphipithecus* was not described until 1937.

As his second line of argument Simons examined the evidence from the deep jaw and the correlated symphyseal characters of *Amphipithecus*. According to him, the ratio of mandible depth to cheek-tooth height in *Amphipithecus* is greater than that of almost all mammals of its tooth size, and "such deep mandibles are characteristic only of certain primates, for example, *Gigantopithecus*, many colobines and some platyrrhine monkeys" and this great mandibular depth is correlated with symphyseal fusion. *Archaeolemur* and *Hadropithecus* are cited as the few lemuriforms with symphyseal fusion; these forms are supposed to have been the lemuriform control experiment. Simons notes that these taxa, for a long time, have been recognized as "independently convergent toward Anthropoidea". There is no mention of Madagascan zoogeography or that these animals, and numerous close relatives, are known from jaws, skulls and skeletons. The recognition from fossils of the lemuriform ties of these animals can hardly be equated with the interpretation of the kind of evidence offered by the three-toothed jaw of *Amphipithecus*.

Simons also states that "the jaws of prosimians, living or fossil, are never particularly deep. The incisors of such prosimians as the lorises and lemurs project forward and there is little or no development of either the superior or the inferior transverse tori at the back of the symphysis connecting and buttressing the two halves of the lower jaw". I should like to counter this statement by commenting that the late Palaeocene *Chiromyoides*, for example, has an exceptionally robust jaw, particularly robust anteriorly, and the ratio of jaw depth to cheek-tooth crown height in this genus is larger than any undoubted catarrhine or platyrrhine, or any other primate. This ratio is 9.0 compared with 5.3 of *Amphipithecus*. *Chiromyoides* also has clearly defined superior and inferior

transverse tori. Unlike the condition of the symphysis in living tarsiers, lorises or lemurs, the earliest known adapid mandibles, those of the North American species of *Pelycodus*, show distinct separation of the superior and inferior transverse tori. Specimens of the medial Eocene adapid *Notharctus* have very robust rami, whereas some fossil catarrhines, the *Parapithecus-Apidium* group for example, have relatively shallow rami. So perhaps the presence of distinct superior and inferior transverse tori and a robust mandible should not be considered as diagnostic criteria for the Anthropoidea, whatever might be included in that suborder.

Simons overlooked the fact that in *Amphipithecus*, a three-premolar primate, the superior transverse torus extends only medially to P<sub>2</sub>, to the line which is medial to the anterior border of P<sub>3</sub>. This condition is similar to that in the adapids, but unlike that in Recent lemuriforms in which the form and function of the anterior lower dentition are greatly altered. But in the two-premolar Oligocene pongids, or in the three-premolar, longer and shallower jawed *Parapithecus-Apidium* group, the superior transverse torus is fully extended medially to P<sub>3</sub>. Those Eocene tarsiiforms in which the symphysis is strengthened by the development of a strong superior transverse torus have this structure extended medially to P<sub>3</sub>.

Among the Eocene tarsiiforms the strong development of distinct superior and inferior transverse tori is frequent and is coupled with a symphysis which was probably immobile. This symphysis was probably derived from a loose, mobile structural type displayed by paromomyids or *Teilhardina*, and it seems to have developed several times among the lemuriforms. Not only do *Archaeolemur* and *Hadropithecus*, as was mentioned by Simons, show fused symphyses, but *Megaladapis*, *Palaeopropithecus*, *Mesopropithecus* and the extant *Lepilemur* show functionally immobile, firmly interdigitating symphyses. *Lepilemur* has a triangular symphysis with an area analogous to the superior transverse torus; *Archaeoindris* has a robustly ankylosed symphysis.

Convergence of *Amphipithecus* to the hominoids is more pronounced than that of any of the large monkey or ape-like Madagascan lemuriforms. This greater similarity is expected, however, because the Eocene lemuroid stock that probably gave rise to *Amphipithecus* did not possess the advanced antemolar homologies or cheek tooth morphology of the Madagascan lemurs, all of which, except possibly the aye-aye, seem to have been derived from a species with a tooth comb and a caniniform P<sub>2</sub>. The Eocene lemurs were dentally much more primitive, with an unspecialized anterior dentition and the tritubercular molars of early Tertiary primates, distinctly closer to the catarrhine ancestors than any of the Madagascan species. Species derived from them therefore would converge more closely with any catarrhine than those derived from a Madagascan stock.

The fossil record will no doubt improve and settle the dust of controversy surrounding *Amphipithecus*. It is of some importance, however, that discussions of divergent views be based on known fossils and on clearly applied and well understood analogies of known evolutionary trends among primates.

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# BOOK REVIEWS

## Testing the Theory of Natural Selection

*Ecological Genetics and Evolution.* Edited by Robert Creed. Pp. xxi+391. (Blackwell: Oxford and Edinburgh, 1971.) £6.50.

For followers of Karl Popper's analysis of science and how it should be done, there is no more dismal example of a metaphysical system masquerading as a science than the theory of evolution. Popper himself, in *The Poverty of Historicism*, singles out evolutionary theory for an attack. "Can there be a law of evolution?" "No, the search for the law of the 'unvarying order' in evolution cannot possibly fall within the scope of scientific method. . .". By this, Popper means only that the history of living organisms and their transformations on Earth are a specific sequence of unique events, no different from, say, the history of England. Since it is a unique sequence, no generalities can be constructed about it. But this aspect of the Popperian objection to biology's most comprehensive system of knowledge is easy to cope with, for uniqueness is in the eye of the beholder. The reason that Popper does not reject the rotation of the Earth on its axis as an unfit subject for science is that, from the standpoint of the celestial mechanic, one day is like another, even if that is not true for the journalist. In like manner, for many purposes, one species is like another, so that for a theoretician of speciation like Ernst Mayr the millions of speciation events that have occurred in the last 500 million years are essentially replications of the same basic sequence of events.

Having disposed of the argument against a scientific theory of evolution in general ("whether in biology or sociology", says Popper) we are faced with a second, and far more serious, problem. There might be scientific laws about evolution, but Darwin's theory of evolution by natural selection in particular is hopelessly metaphysical, according to the rules of etiquette laid down in *The Logic of Scientific Inquiry* and widely believed in by practising scientists who bother to think about the problem. The first rule for any scientific hypothesis ought to be that it is at least possible to conceive of an observation that would contradict the theory. For what good is a theory that is guaranteed by its internal logical

structure to agree with all conceivable observations, irrespective of the real structure of the world? If scientists are going to use logically unbeatable theories about the world, they might as well give up natural science and take up religion. Yet is that not exactly the situation with regard to Darwinism? The theory of evolution by natural selection states that changes in the inherited characters of species occur, giving rise to differentiation in space and time, because different genetical types leave different numbers of offspring in different environments. In particular, a species of bird with a small bill may evolve a larger bill size, because some aspect of the environment has changed so that large billed birds now have more offspring. Or the species may split into two new contemporaneous species with different bill sizes, because geographically separated populations of the original species lived in different environments in one of which large-billed birds were more fecund, while in the other small-billed birds left more offspring. Such a theory can never be falsified, for it asserts that some environmental difference created the conditions for natural selection of a new character. It is existentially quantified so that the failure to find the environmental factor proves nothing, except that one has not looked hard enough. Can one really imagine observations about nature that would disprove natural selection as a cause of the difference in bill size? The theory of natural selection is then revealed as metaphysical rather than scientific. Natural selection explains nothing because it explains everything.

The trouble with this analysis is that even though natural selection might not be an epistemologically satisfactory hypothesis, it might nevertheless be true. Very inconvenient, but there you are. The way out, taken by evolutionists, is to go back to older ideas of confirmation of hypotheses, rather than Popperian falsification, and to notice that there are two different sorts of theories, with respect to confirmation, that are indistinguishable from the point of view of falsification. The first sort, typified by an appeal to a supernatural creator or omnipotent god of some kind, is not only not falsifiable, but every observa-

tion about nature is a positive confirmation and necessarily so. "The heavens declare the glory of God: and the firmament showeth his handiwork", says the psalmist. The second sort, to which Darwin's theory of natural selection belongs, although not falsifiable because not universally quantified, could fail of confirmation in any number of cases, indeed always. So if we chose 100 examples of variation between organisms in space or time and tried to pin down the environmental circumstances responsible, we might succeed, after immense effort, in producing only two reasonably convincing cases. Then we would be in a Popperian pickle because we could not know whether natural selection was in fact a rare cause of evolution, or whether it was a common cause but damned hard to demonstrate. Everyone would have wasted his time. But suppose, instead, that ninety-eight good confirmations resulted. Then, if the cases were chosen without bias, every reasonable person would be forced to admit the rule of natural selection amid joyous cries of "No Poppery!".

It is no wonder, then, that many evolutionists have devoted their efforts to revealing the operation of natural selection in a variety of plants and animals. It has been their hope to explain so many cases of spatial and temporal variation, and presumably to leave such a small residue of unsubstantiated cases, as to convince themselves and everyone else that natural selection is the chief agent in determining evolutionary change. In very large part this has been a British pastime, traceable to the fascination with birds and gardens, butterflies and snails that was characteristic of the prewar upper middle class from which so many British scientists came. E. B. Ford, the social and scientific quintessence of that tradition, was one of the earliest to devote his attention to the demonstration of natural selection. *Ecological Genetics and Evolution* is his Festschrift and it is replete with primroses, snails, ladybirds and the Pale Brindled Beauty Moth. Like many such Festschriften, it is a collection of research papers with a few general reviews interlarded. The reader interested in general evolutionary problems will find the



review articles useful; on the whole it is a book that should be in all institutional libraries but probably not in private collections.

The papers in this collection show the attempts in a variety of organisms to give a causal explanation of genetic polymorphisms in terms of differential rates of survival and reproduction of different genotypes. The issue, it seems to me, is in doubt. There have been great successes, as for example the cases of Müllerian and Batesian mimicry in moths and butterflies so brilliantly worked out by the Browsers and Phillip Sheppard (who unaccountably is not represented by an essay). Natural selection not only operates, but works to make tasty butterflies look like nasty ones, and nasty ones to look like each other. And then there are the persistent failures, like the extraordinary diversity of human blood-group polymorphisms which show barely a trace of differential fecundity and mortality, despite an immense collection of data on the world's best studied species. Indeed, the only really convincing case of differential mortality in a blood group, that for Rh incompatibility, operates in the wrong direction!

To understand the bulk of the evidence about natural selection we must distinguish between what we may call "tautological" selection and "functional" selection. The difference can be illustrated by the work on industrial melanism in moths. Ford, Kettlewell, Clarke and Sheppard have demonstrated the undoubted protection that melanism offers against visual detection by predators in polluted areas (functional selection), but also that melanic genotypes in heterozygotes, or in larvae where the coloration is not at issue, simply leave more offspring (tautological selection). In functional selection the character itself is of advantage to the organism in its relation with physical or biotic factors of the environment. In tautological selection the character is accidental (or even not yet present, as in larvae) and some other physiological effect of the gene, unseen by the observer, must be postulated to account for the geographical or temporal variation. Indeed it is often possible to show that different genotypes really do leave different numbers of offspring in different environments, as for different sized animals in *Cepaea*, yet no "explanation" is known. But if the spots on ladybirds are black in industrial areas and red in unpolluted areas, not because of the selective advantage of the colour itself, but because the enzyme that works better in polluted areas happens to give black pigment, then only in a formal and rather trivial sense is spot colour evolving because of natural selection. It will be a hollow triumph indeed for those who see

natural selection as all powerful if it turns out that a very large proportion of morphological and behavioural evolution is nothing but the accidental concomitant of selection of polypeptides with greater heat stability. For it will destroy the chief purpose of all the studies on polymorphism described in *Ecological Genetics and Evolution*, which is to make such a clear case for the generality of functional selection that we will be able to trust functional explanations in general. Natural selection of the character states themselves is the essence of Darwinism. All else is molecular biology. R. C. LEWONTIN

## Staudinger's Memoirs

*From Organic Chemistry to Macromolecules: a Scientific Autobiography based on My Original Papers.* By Hermann Staudinger. Translated from the German. Pp. xvi+303. (Wiley Interscience: New York and London, January 1971.) £7.

PROFESSOR HERMANN STAUDINGER will be best remembered as one of the founders of macromolecular chemistry. He was an outstanding organic chemist and in 1961, at the age of 80, he published in Germany his *Arbeits Erinnerungen*. Now, some ten years later, we have the English translation under the very appropriate title *From Organic Chemistry to Macromolecules*. This book is much more than a catalogue of his scientific achievements. It vividly illustrates the breadth of vision which informed the pioneering work which was to lead to modern concepts of the mechanism of polymerization and the structure of vinyl compounds. We read in his own words how his views and concepts developed and how the macromolecular hypothesis emerged from a sea of conflicting views.

Before Staudinger embarked upon his studies of polymerization in the 1920s he had been concerned with the typical problems of classical organic chemistry with its well-defined substances of low molecular weight which could be characterized by well-established methods. His scientific works fall naturally into the two divisions dealing with these two aspects of his research. During the period from his first research post with Vorlander at Halle in 1903 until he moved from Zurich to Freiburg in 1926 he worked on many problems of synthetic organic and natural product chemistry. His most important contributions were to the chemistry of the ketenes. This work and his excursions into synthetic drugs, insecticides, the aroma of coffee, and so on, together with many personal recollections of his teaching work, his colleagues and leading German chemists with whom he

came into contact, are the subject of the first part of his book.

Staudinger did not begin his main work on the substantiation of the macromolecular theory until he left Zurich, and the decade 1920-30 was the vital period in his fight for what was, in his own words, "a new branch of organic chemistry". In 1920 he began his studies on the structure of macromolecular compounds, in particular polyoxymethylene, polystyrene and natural rubber, and in an important paper published in that year he deplored the prevailing tendency to formulate polymeric substances as "association compounds". He proposed in this paper the chain formulae now accepted for polystyrene and polyoxymethylene. He also advocated the then radical point of view that natural rubber was a long chain molecular structure. In the years that followed Staudinger relentlessly championed the molecular or primary valence structure of high polymers or macromolecules as he later chose to call them. His views were not widely accepted and disputes with his critics were many and often. His battles against the opponents of his theories on the one hand, and with those who differed from him in detail rather than in principle on the other, led him to considerable experimental work in support of his views. By 1926, however, most of the principal problems were resolved and his macromolecular hypothesis received wide acceptance. The firsthand account of the whole story from Staudinger's point of view is clearly told in the annotated and edited excerpts in his book. The story as told of the development of the macromolecular hypothesis is an account of the making of chemical history and the establishment of a branch of organic chemistry which has become more important than its principal founder could have imagined. Staudinger was awarded the Nobel Prize for his work in 1953, and the text of his Nobel Lecture, which is reproduced in these memoirs, is a notable statement and a clear summary of his outstanding contribution to chemistry. All readers of this volume will be grateful to Professor and Mrs Staudinger for this historical review of the beginnings of macromolecular chemistry. C. E. H. BAWN

## Spectroscopic Devices

*Spectroscopy and its Instrumentation.* By P. Bousquet. Translated by K. M. Greenland. Pp. viii+239. (Adam Hilger: London, 1971.) £7.

THE French original of this book, *Spectroscopie Instrumentale*, which was published in 1969, was based on a course of advanced optics for students in the Faculty of Science of the Uni-

versity of Aix—Marseille. It describes in detail optical instruments and devices used in spectroscopy, ranging from the prism spectrometer to apparatus for Fourier transform methods, and is the first book, as far as I know, which covers the whole field. In the translation opportunity has been taken to add a short account of recent developments in the use of interferograms as diffraction gratings.

A short, introductory chapter is used to establish the significance of terms such as radiance, resolving power and luminosity, and these together with the conception of instrumental profile are then used extensively in subsequent chapters in the relative evaluation of different instruments and techniques. This is a very unconvincing chapter, possibly because the author avoids the use of units, but fortunately it is not an indication of the level of the remainder of the book.

The design, properties and limitations of prism and grating spectrographs are thoroughly discussed, and a chapter is devoted to the production and properties of gratings, including a brief account of the Merton-NPL process. In the latter the description of the preparation of a usable grating from a helical ruling is misleading: the pellicle removed from the cylinder is not itself mounted on glass and used as a mould (like a Thorp replica), but its pattern is impressed into the surface of a gelatine coated blank. In the early days of the process this gelatine grating after hardening was the final product, but later more durable gratings were made in Marco resin using the gelatine "master" as a mould. Contrary to the opinion expressed, in the spectral region for which they are intended these replica gratings compared favourably with the products of the interferometrically controlled flat-bed ruling engine, and still do. A short account is also given of the preparation and properties of "holographic" gratings: is the use of this term (elsewhere, as well as in this book) really justified? The idea of using interferograms as diffraction gratings goes back at least as far as Rayleigh, and their actual use at least as far as Burch! The big step forward has been due to the advent of the laser as a powerful, coherent source and to the use of photo-resist materials for recording the interference patterns.

Prism and grating spectrometers and monochromators are described in detail, and their properties discussed and compared with those of spectrographs; here the term instrumental profile is defined and its significance indicated. The principle of the grill spectrometer is described, and its advantage of increased *étendue* is outlined. The remaining third of the book is concerned with interference spectroscopy, commencing with a lengthy account of the

Fabry-Perot étalon spectrometer. A few pages are devoted to the Sisam interferometer of Connes, and finally there is an account of the use of the Michelson interferometer in Fourier transform spectroscopy.

In content the translation differs only a little (as indicated above) from the French original; the latter has a much more pleasing format and typeform, but the diagrams in the translation are greatly improved—they appear to have been generally re-drawn—and the few plates are vastly superior (it is a pity, however, that their connexion with the relevant text could not have been made more obvious).

The translation has been excellent; the book appears to have been virtually re-written in English, which is as it should be. I can really find only two points of contention. The first is perhaps trivial: for "objective de chambre" why use "focusing lens" instead of "camera lens"? The second is of importance, because the English title is misleading: this book is concerned with the instruments used in spectroscopy, and not with the subject of spectroscopy itself and the French title makes this clear. Of course, spectroscopists will find it exceedingly useful; one can always get the best out of instruments with a knowledge of how they work, and this is probably more true of spectroscopic instruments than of any others, because of the frequent individuality of the problems to which they are applied.

K. J. HABELL

## Computers and People

*Computers and Crisis: How Computers are Shaping Our Future.* Edited by R. W. Bemer. Pp. 401. (Association for Computing Machinery: New York, 1971.)

UNLIKE the usual proceedings of technical conferences, this is a condensed report, which summarizes the principal points raised by the numerous contributors in the 115 sessions of the Conference of the Association for Computing Machinery in 1970.

The report was prepared in such a manner that . . . "the result would be a readable volume for the generalist, requiring a minimum level of knowledge of, or contact with, the technical computer industry". To this end, Mr Bemer has to be congratulated for his efforts.

The conference sessions were categorized into seventeen sectors of interest. These sectors were further subdivided into special topics which were discussed in separate sessions. The entire list cannot be included here for obvious reasons. Some representative examples are: the sector on "Industry", which included sessions on "Food Dis-

tribution", "Plant Automation", and "Printing and Publishing" amongst others; the sector on "Education", including "Computer Science Education" at the general, undergraduate and graduate levels; and finally, the sector on "Finance", including sessions on "Securities", "Insurance", "Banking" and "Accounting".

Although the topics cover a wide range of application areas, there were comments throughout the report on aspects of the computing environment which were of common concern. These were primarily on the use of computer networks and data banks. More specifically, the questions raised frequently concerned the reliability, privacy and security aspects of computer networks and data bases.

The observations, comments and opinions recorded were not only from the computer scientist or technologist, but also from people of different disciplines, whose common attribute was to have been near, or had access to, a computer. To stress the extent of the diversity, a selection of the participants is given here.

An FBI special agent commented on the effectiveness of the National Crime Information Center in law enforcement. A consultant, who is an ex-convict, described the plans for computer training courses for convicts as part of rehabilitation programmes. Finally, a representative from the US General Accounting Office discussed the introduction of computer technology into Congress, that is, into the processes of government. One interesting comment here was that Congressmen may have to write computer programs to assist them in their routine operations.

This volume should be read by all who are interested in how computers are shaping our future. Whether the predictions contained within will come true is a question for future historians, who will find this volume an invaluable source of information. A. L. LIM

## Liquid Crystals

*A Review of the Structure and Physical Properties of Liquid Crystals.* By Glenn H. Brown, J. W. Doane and Vernon D. Neff. Pp. 95. (Butterworth: London, September 1971.) £5.

THIS review appeared in its present form as one of the *CRC Critical Reviews in Solid State Sciences* (Volume 1, Issue 3, September 1970). An appendix listing papers presented at the Third International Liquid Crystal Conference, Berlin, Germany, 1970, has, however, been added, together with some references on diffusion in liquid crystals. The review covers literature on liquid crystals mainly from



January 1960 to February 1970. Even so, because of the rapidly developing nature of the subject, the authors have had to be selective in the areas covered. As authorities in the field, they have, however, chosen judiciously. The review begins with a brief survey of the geometry of the molecules of compounds which form thermotropic liquid crystals, and moves on to a useful review of the very active areas concerning polymorphism of liquid crystals and theories of liquid crystal structure. This is followed by a survey of recent work on certain physical properties and physical methods of studying thermotropic liquid crystals, attention being paid mainly to effects of external forces and fields, thermodynamic behaviour, nuclear magnetic resonance spectroscopy, electron paramagnetic resonance spectroscopy, Mössbauer effects, light scattering and spin-lattice relaxation. Infrared and Raman spectroscopy receive only brief mention, as do some other physical properties such as viscosity, ultrasonics, Brillouin scattering, positron annihilation and birefringence.

A most attractive feature of the review is that it ends with an eleven page section on lyotropic liquid crystals, that is, on liquid crystals formed by amphiphilic compounds and solvents. Too often the so-called thermotropic and lyotropic classes of liquid crystal are divorced from one another in reviews and texts on liquid crystals, whereas ideally the two should be considered side by side so that developments in one area may stimulate progress in the other. This section is most informative, and the diagrams of the structures of the various lyotropic mesophases are particularly useful.

This hard backed version of the original article is well written and attractive in its form and presentation. It should be stressed, however, that it is not a textbook on liquid crystals, and that because it is a selective review of particular areas, some topics have not been covered. One of these is the technological applications of liquid crystals. The book will therefore appeal mainly to those engaged on pure research on liquid crystal systems.

G. W. GRAY

## Nuclear Apple

*The Nuclear Apple: Discoveries in Fundamental Physics.* By P. T. Matthews. Pp. viii+150. (Chatto and Windus: London, 1971.) £2.00.

In this book, Professor Matthews describes developments in fundamental physics which have led to our present state of understanding and lack of understanding of sub-nuclear or elementary particle physics. The book is aimed at the non-specialist and the language

is simple, direct, and generally free from technical jargon.

Both the title and the sub-title are something of a puzzle, since many topics in fundamental physics are omitted or given only passing reference, and the physics of nuclei is dismissed with the observation that in a typical nucleus "the situation is far too complicated to be sorted out in any detail". However, such topics as the nature of the strong and weak interactions and the classification of particles are discussed with insight and vivid analogy. Most important, perhaps, are the pages devoted to discussion of the function of physics, its relation to mathematics, and of the role of observation and prediction in a quantum theory.

From a pedagogical point of view, it seems a pity that the symbol  $h$  has been used to represent  $h/2\pi$  so that the spin of the electron appears as  $\frac{1}{2}h$  and the energy of a photon is written as  $E=h\omega$ . Also, a surprisingly concrete picture is given of the orbital motion of an electron in an atom and of the spin-angular momentum.

The comments of a reviewer are to a large extent irrelevant for a book of this type. What matters is the response of the non-specialist reader. It is to be hoped that this book will find its way into many school libraries and that it will make the sort of impact that the works of Jeans, Eddington, and Andrade have made on previous generations.

DAPHNE F. JACKSON

## Electrical Machines

*Linear Electric Motors.* By E. R. Laithwaite. Pp. 101. (Mills and Boon: London, October 1971.) £2.50.

PROFESSOR LAITHWAITE is a superb popularizer and is incapable of writing a dull book; this is no exception. *Linear Electric Motors* is a short book (101 pages), which deals with a wide range of topics. It is thought-provoking, but I am forced to ask, who Professor Laithwaite had in mind when he wrote it? In spite of the apparent simplicity of his writing, Professor Laithwaite implicitly assumes a substantial knowledge of conventional electrical machines, so that the inexperienced reader who wants a broad-brush treatment of linear motors would find himself in deep water at times. If he is prepared to skip the profound passages, which are sketchy because of the brevity of the book, he will get a fair picture of what can be done with linear machines and a challenging picture of where imaginative topological tricks might lead electrical machines. He will be less well served in discussion of what a linear machine cannot do, which may be his reason for reading the book!

On the other hand, the book draws so

extensively on the author's more scholarly "Induction Machines for Special Purposes" (Newnes, 1966) that the informed reader has a strong sense of "déjà vu" and, on checking, finds that many of the diagrams (and many of the ideas) previously appeared in that book, but with a much more thorough explanation. Yet the earlier book is not quoted in the main text and only appears as item 13 in the references. It is hard to believe the statement on the cover that the work "contains the most complete and up-to-date information . . . so far published"; for those "skilled in the art", the earlier work is more complete and essentially up-to-date, and so is to be preferred.

E. J. DAVIES

## Diffraction from Gases

*Electron Diffraction in Gases.* By Michael I. Davis. Pp. x+181. (Marcel Dekker: New York, 1971.) \$12.50.

SINCE our boyhood at Latymer Upper School, Michael Davis and I, entirely by coincidence, have found our paths crossing repeatedly as researchers in gas-phase electron diffraction. Workers in the field number no more than about fifty, worldwide, in spite of the extremely valuable information about molecular geometry which the method produces. Davis traces the origins of the specialized modern techniques to the equipment and philosophies of the Michigan and Norwegian groups of the 1950s. Perhaps he underrates the contributions of the Russian and Japanese groups, but most students in the field still make their pilgrimages to Ann Arbor and Oslo. His experience with Norwegian and American groups equips him well to write this book—the first devoted wholly to the subject. The book is oriented towards the (graduate) student; it will serve as an excellent introduction to the method, but lacks illustration of its applications and contains the vagaries often associated with students' books. One drawback to this approach is that many concepts and formulae must be stated without proper justification or derivation; this certainly shortens the book, but one is in danger of producing graduates who, like some of Asimov's inhabitants of the future, are unable to do more than follow the rituals laid down by their predecessors. But taking into account the pressures placed by publishers on authors these days—to be brief yet still comprehensive—I am sure this would have been just the sort of book I would have written myself had I not been deflected from the task by a move from Glasgow to Manchester. I shall certainly recommend the book to my specialist students.

BRIAN BEAGLEY

# Obituary

## Dr Vikram A. Sarabhai



In the early hours of December 30, 1971, Dr Vikram Ambalal Sarabhai, chairman of India's Atomic Energy Commission, died unexpectedly of a heart attack at Trivandram in South India. He was 52 years old.

Dr Sarabhai was a well known scientist working in the area of cosmic rays, particularly in the use of the cosmic-ray signal for the study of interplanetary phenomena. At the time of his death, besides being chairman of the AEC he was also director of the Physical Research Laboratory, Ahmedabad, chairman of the Indian Space Research Organization and director of the Space Science Technology Centre at Thumba.

Dr Sarabhai was born on August 12, 1919. His early education was at Ahmedabad, but later he went to Cambridge and took his Tripos in Natural Sciences in 1939 at St John's College. He started his professional career at the Indian Institute of Science, Bangalore, with Professor Sir C. V. Raman. There he wrote his first paper on testing the randomness of the arrival time distribution of cosmic-ray particles. After the war he returned to Cambridge to work for his PhD, which he received in 1947. Soon after his return he founded the Physical Research Laboratory at Ahmedabad. Most of his later scientific work was done at this laboratory, where he built up excellent schools of cosmic-ray physics, space science, upper atmosphere and ionosphere physics, theor-

etical nuclear physics and plasma physics.

In the field of cosmic-ray variations, along with a number of his colleagues, he made several significant contributions. He was one of the first people to realize, through experiment, that the variations of the muon component could not be understood only in terms of atmospheric effects but, in fact, implied an anisotropy of cosmic rays in interplanetary space. He pointed out that by working at near-equatorial stations his measurements would be particularly sensitive to the anisotropy of cosmic rays in the plane of the ecliptic. His work showed that this anisotropy changed with the 11-year cycle of solar activity.

The special object of the work of Dr Sarabhai and his collaborators at Ahmedabad was to identify the electromagnetic conditions in interplanetary space with measurements of cosmic-ray intensity and disturbances in the geomagnetic field made on the surface of the Earth. In 1963 Dr Sarabhai suggested that the variation in the intensity of the green coronal emission line implied a variation in the velocity of the solar wind emitted from different regions of the Sun. He was also probably the first to describe the interesting interplanetary and geomagnetic phenomena resulting from special electromagnetic configurations produced by the interplay of fast and slow streams of plasma emanating from the Sun. On the assumption that periodic fluctuations in the horizontal component of the Earth's magnetic field are due to the large scale magnetic field inhomogeneities in the solar plasma impinging on the magnetosphere and by analysing the period of these fluctuations, he and his collaborators suggested that the average scale size of these inhomogeneities is 0.02 a.u.

Using large counting rate telescopes, some of them pointing north, he initiated a study of interplanetary conditions not only in the plane of the ecliptic but also at high heliolatitudes. Later he and Dhanju set up a very large counting rate muon telescope at Chacaltaya to study fluctuations in the dynamical range 1 to 30 cycles<sup>-1</sup>, and they were able to show that there is a complete correspondence between the peaks in the frequency spectrum of the cosmic-ray fluctuations and that of the fluctuations in the magnetosphere and inter-

planetary space observed by satellites.

Dr Sarabhai's more recent work was connected with an analysis of geomagnetic fluctuations and their interrelation with phenomena occurring in the atmosphere, the ionosphere, the magnetosphere and also in interplanetary space in response to solar activity. He used to say that it was almost possible to throw away the satellites and study what is going on in interplanetary space through an analysis of phenomena on the Earth.

Valuable as Dr Sarabhai's contributions were to the scientific field in which his primary interest lay, he will be remembered in his own country for his continuous involvement in bringing modern science and technology to bear on the important problems of India. Thus as chairman of the AEC he enthusiastically subscribed to and furthered Homi Bhabha's belief that a nuclear power programme would be at least as important for some developing countries as for industrialized countries. He formulated plans for agro-industrial complexes built around large nuclear power plants, which would serve as food factories in addition to providing the power for large industrial enterprises. His interest in space grew naturally out of his preoccupation with natural phenomena in interplanetary space; however, he pushed his plans for communication and television satellites with a missionary zeal because he believed that this would perhaps be the only way in which the half million villages of his country could be knitted together, and effective programmes in family planning, adult education and agricultural reform launched.

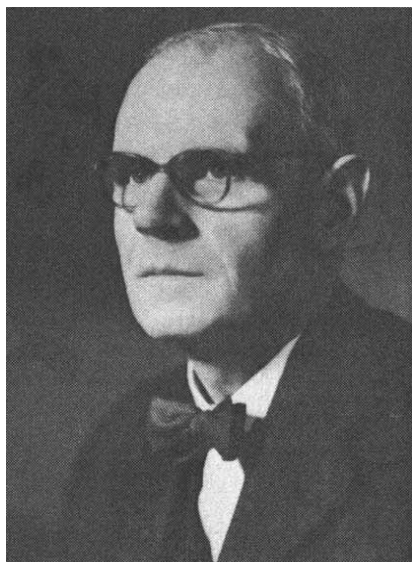
He had a special aptitude for handling men and organizations. In the traditional structure of India he emphasized the importance of the managerial role of scientific and technical men for converting responsibilities for mere control into functions requiring innovation and achievement. Thus alongside his scientific and technological activities he had time and energy to help set up the Ahmedabad Textile Industries Research Association and the Indian Institute of Management and a Community Science Centre at Ahmedabad. Dr Sarabhai's humanistic interests led him to an active participation in the Pugwash Movement and in large scale problems of disarmament and of development.



In his style of working, hurry had a charming leisurely quality about it, even though every minute of his time was claimed by one or the other of his self-created projects. Within the space of an hour he could go from cosmic-ray modulation, to rocket propellants at Thumba, to power reactors at Kalpakam, to the television satellite experiment, to community science education, to antenna systems for communication links, to infrared devices for surveillance of crop disease, to the administrative details of setting up a new public corporation and back perhaps to cosmic rays. And each of these transitions was deep, involving decision making, un wasteful of words, and outwardly done with a naturalness and charm which always overwhelmed his friends.

Vikram Sarabhai will be missed on the Indian scene, as a scientist, as a scientific visionary, as a doer of things and as a remarkable human being.

## Sir Charles Harington



WITH the death of Sir Charles Harington on February 4, Britain lost not only an outstanding biochemist who was one of the leading figures in the field between the two wars, but also a distinguished administrator of medical research.

Charles Robert Harington was born in 1897 in that part of Herefordshire which is close to the Welsh border. His father was a parson with strong scholarly leanings and his general family background was that of law and the army rather than of science. From Malvern College he went to Cambridge where he read chemistry. A physical disability prevented Harington from joining one of the services in the first war and he was thus able to complete his degree. From Cambridge he went

to Edinburgh to work under Barger, who exerted a profound influence on his scientific development. For the rest of his life Harington became identified with biological and particularly medical research, and his knowledge of medicine and his special understanding of clinical problems dated largely from his early experience in Edinburgh. After a short stay in the United States, where he worked with H. D. Dakin and D. D. Van Slyke, he went to University College Hospital Medical School in London, where he spent twenty happy years. In 1942 he was appointed director of the National Institute for Medical Research which, at the time of his appointment, was situated at Mount Vernon, Hampstead. The staff of the institute at that time was relatively small and a large part of the work which went on was either directly or indirectly connected with the war effort. At the end of the war some new appointments to the scientific staff were made, but no great expansion was possible until, in 1949, the institute was moved to its present site in Mill Hill. Harington was mainly responsible for the considerable development of the National Institute which took place at that time.

The appointment of Harington, a non-medical man, as director of the National Institute for Medical Research was somewhat unexpected, but he fully justified the confidence which the Medical Research Council placed in him. One of his outstanding characteristics was the unfailing support and wise counsel which he gave to promising young research workers. There are many people who now occupy important positions in biomedical research in Britain who owe their success to a large extent to the help Harington gave them early in their careers. He also considered it important to establish close contact between the different divisions and so any attempt to over-emphasize departmental boundaries was resisted. This planned cooperation between different disciplines, he considered, was one of the major differences between a national institute and the set-up of ordinary university departments. On the other hand, Harington was careful not to interfere with the scientific freedom of his staff, and this was so marked that it was sometimes difficult to obtain any advice from him on scientific matters. Harington felt that a research institute provided the opportunity for gifted people to concentrate on research and be free from major administrative responsibilities. It was for this reason that he found it difficult to delegate managerial responsibility to a significant extent, even to his senior colleagues. Harington managed to attract many outstanding workers to the National Institute

and he maintained the standard of excellence which was set up in the first place, for a much smaller institute, by Sir Henry Dale.

The scientific work for which Harington will best be remembered is that which he published in 1926 and 1927 on the chemistry of thyroxine. He established the structure of this hormone and together with Barger described its synthesis. Two years later he succeeded in resolving thyroxine and he also characterized other iodine-containing compounds of the thyroid gland. It is perhaps difficult nowadays to appreciate fully the excitement which was caused among British biochemists when Harington first announced the structure of thyroxine at a meeting of the Biochemical Society in 1926.

In 1935 he synthesized glutathione, which at that time was an impressive achievement. A few years later Harington started a series of investigations in the field of synthetic immunology, in which specific antibodies were used to examine certain metabolic processes. He was, for instance, able to suppress the action of thyroxine in rats by prior treatment with anti-thyroxyl thyroglobulin serum. This work, which involved the preparation of well-defined derivatives of proteins, was interrupted by the Second World War. Harington also worked on insulin, on the synthesis of peptides and various other topics. Techniques he used were largely those of the classical organic chemist, but his general outlook was that of a biologist. After the Second World War, Harington started experimental work again in the laboratory, but because of his heavy administrative duties he found it difficult to acquire the new techniques which were being introduced at that time. He preferred to work mainly with his own hands and never aspired to leading a large team.

Harington had a profound influence on a large number of medical scientists and biochemists, and was greatly respected in the scientific world generally. He was possessed of an unusually marked sense of duty and he was a man of considerable loyalty and deep affection, which he was very shy to display. He was a member of the Medical Research Council, a member of the Agricultural Research Council, an honorary secretary of the Biochemical Society and editor of the *Biochemical Journal* for about 12 years. Among the many honours he received was the KBE in 1962, the Royal Medal of the Royal Society in 1944 and the Gold Medal of the Society of Apothecaries in 1953. What he valued more than any honours which were bestowed on him, however, was the affection and respect which he received from almost everyone who was closely associated with him.

# Announcements

## University News

**Dr Graham Wood** has been appointed professor of corrosion science in the **University of Manchester Institute of Science and Technology**.

**Professor C. A. Coulson** has been appointed first professor of theoretical chemistry in the **University of Oxford**.

**Dr D. T. Elmore** has been appointed to the J. C. White chair of biochemistry in the **Queen's University of Belfast**, in succession to **Professor A. G. Lloyd**.

## Appointments

**Professor John Nisbet**, University of Aberdeen, has been appointed a member of the **Social Science Research Council**.

**Professor F. H. Hahn**, London School of Economics and Political Science, and **Dr D. T. N. Williamson**, Molins Machine Company Limited, have been appointed to the **Council for Scientific Policy** in succession to **Professor H. G. Johnson** and **Dr G. B. R. Feilden**.

## Miscellaneous

The following have been elected **Fellows of the Royal Society**: **Professor M. L. Barr**, University of Western Ontario; **Dr J. S. Bell**, CERN; **Dr B. J. Birch**, University of Oxford; **Dr D. M. Blow**, MRC; **Professor G. Bond**, University of Glasgow; **Professor G. V. R. Born**, University of London; **Dr S. F. Boys**, University of Cambridge; **Dr W. Bullerwell**, Institute of Geological Sciences; **Mr D. P. Burkitt**, MRC; **Dr V. E. Cosslett**, University of Cambridge; **Professor A. Dalgarno**, Harvard University; **Dr F. J. M. Farley**, Royal Military College of Science; **Dr G. Fryer**, Freshwater Biological Association; **Dr R. M. Gaze**, MRC; **Professor V. Gold**, University of London; **Dr J. A. B. Gray**, MRC; **Professor A. J. Haddow**, University of Glasgow; **Dr D. K. Hill**, University of London; **Dr J. T. Houghton**, University of Oxford; **Professor A. Keller**, University of Bristol; **Professor H. Lehmann**, MRC; **Sir Morien Morgan**, Royal Aircraft Establishment; **Professor W. D. Ollis**, University of Sheffield; **Dr B. P. Pal**, Indian Council of Agricultural Research; **Dr Mary Parke**, Marine Biological Association; **Professor R. Penrose**, University of London; **Professor G. H. Rawcliffe**, University of Bristol; **Professor A. E. Ringwood**, Australian National University; **Dr R. A. Sanger**, MRC; **Professor F. J. Ursell**, University of Manchester; **Dr R. J. P. Williams**, University of Oxford; **Dr C. A. J. Young**, Imperial Chemical Industries Limited.

The **William Bate Hardy prize** of the **Cambridge Philosophical Society**, awarded once every three years, has been won by **Dr Enid A. C. MacRobbie**, Girton College, for her work on biophysical plant physiology.

The **International Health Foundation** will award a prize of 5,000 Swiss francs for the best study submitted on the theme "Prospects for the practical application of prostaglandins". A further award of 2,000 Swiss francs may be made for a paper submitted by an author under 35 years of age. Further information can be obtained from the International Health Foundation, 1 Place du Port, 1204 Geneva, Switzerland.

The **Societas Internationalis Odonatologica**, founded during the first European symposium on odonatology held in Ghent last year, will publish an international journal, *Odonatologica*, to perpetuate contacts between workers in the field and to promote research. The journal will contain original research papers and preliminary notes in English, French, German and Italian and will appear quarterly. Further information about the society can be obtained from the treasurer, and about the journal from the editors, Societas Internationalis Odonatologica, Institute of Genetics, University of Utrecht, Opaalweg 20, Utrecht, The Netherlands.

**Dr Sheina M. Marshall**, recently of the Scottish Marine Biological Association Marine Laboratory, has been awarded the Neill Prize of the **Royal Society of Edinburgh** for her contributions to marine biology. The Society's Keith Prize has been awarded to **Dr Charles D. Waterston**, Royal Scottish Museum. The following have been elected to fellowship of the Society: **Dr L. W. Barr**, Paisley College of Technology; **Mr B. T. Bellis**, Daniel Stewart's College; **Professor J. G. Buchanan**, Heriot-Watt University; **Professor D. S. Butler**, University of Strathclyde; **Professor R. A. Cowley**, University of Edinburgh; **Professor A. E. H. Emery**, University of Edinburgh; **Professor D. S. Falconer**, University of Edinburgh; **Professor R. B. Goudie**, University of Glasgow; **Sir Norman Graham**, Scottish Education Department; **Professor F. D. Gunstone**, University of St Andrews; **Professor W. M. Hutchison**, University of Strathclyde; **Professor A. Jeffrey**, University of Newcastle upon Tyne; **Dr M. R. W. Johnson**, University of Edinburgh; **Professor M. S. Laverack**, University of St Andrews; **Dr S. G. G. MacDonald**, University of Dundee; **Professor E. M. McGirr**, University of Glasgow; **Professor J. R. Mallard**, University of Aberdeen; **Professor B. P. Marmion**, University of Edinburgh; **Professor J. L. Monteith**, University of Nottingham; **Dr J. W. Porteous**, University of Aberdeen; **Professor W. E. Spear**,

University of Dundee; **Dr P. N. R. Usherwood**, University of Glasgow; **Dr A. B. Wallace**, University of Edinburgh; **Professor A. C. Wardlaw**, University of Glasgow; **Professor M. B. Wilkins**, University of Glasgow; **Professor C. W. M. Wilson**, Trinity College, Dublin; **Professor P. A. H. Wyatt**, University of St Andrews.

## International Meetings

**April 12, Inaugural Meeting of the British Transplantation Society**, London (Professor L. Brent, Department of Immunology, Wright-Fleming Institute, St Mary's Hospital Medical School, London W2).

**April 13, Computer-assisted Analysis of Complex Synthetic Problems** (Ciba Foundation Lecture), London (Personal Assistant to the Director, The Ciba Foundation, 41 Portland Place, London W1N 4BN).

**April 25, Flight Deck Displays and Instrumentation for Future Civil Short-field Aircraft**, London (Institution of Electronic and Radio Engineers, 8-9 Bedford Square, London WC1).

**April 27, Trends in Electroanalysis**, London (Society for Analytical Chemistry, 9-10 Savile Row, London W1X 1AF).

**April 29, Routes to Qualification in Mathematics**, London (Secretary and Registrar, Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend on Sea, Essex SS1 2JY).

## Errata

IN the article "A New Approach to Pattern Recognition" by N. Jardine (*Nature*, **234**, 526; 1971), the second sentence of the third paragraph should read as follows: "It is assumed that each of a set of classes,  $\omega_1, \dots, \omega_n = \Omega$ , has an *a priori* probability of occurrence  $P(\omega_i)$  and is specified by a conditional probability density function  $p(X|\omega_i)$ ,  $i = 1, \dots, n$ , over attribute states,  $x_1, \dots, x_m = X$ ". In the third sentence of the fourth paragraph " $\omega$ " should be " $\omega_1$ ".

The entry on the contents page for the article "Suppression of the Immune Response: Reversal of the Disease State with Antigen in Allergic Encephalomyelitis" by E. H. Eylar, J. Jackson, B. Rothenberg and S. W. Brostoff (*Nature*, **236**, 74; 1972) should have read:

**Immune response**—Allergic encephalomyelitis reversed by antigen treatment—EYLAR, JACKSON, ROTHENBERG and BROSTOFF (Merck Institute).



# Reports and Publications

not included in the *Monthly Books Supplement*

## Great Britain and Ireland

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 263, No. 850 (6 January 1972): Ordovician Geography and Faunal Provinces Deduced from Trilobite Distribution. By H. B. Whittington and C. P. Hughes. Pp. 235-278. (London: The Royal Society, 1972.) £1.15; \$3. [121]

Flora of Tropical East Africa. Edited by E. Milne-Redhead and R. M. Polhill. Rhamnaceae. By Marshall C. Johnston. Pp. 40. (London: Crown Agents for Oversea Governments and Administrations, 1972. Published on behalf of the East African Community.) 35p. [131]

Agricultural Research Council. Annual Report of the Meat Research Institute, 1970-71. Pp. 105. (Langford, Bristol: Meat Research Institute, 1972. Obtainable from HMSO.) £1 net. [131]

Freedom From Hunger Campaign. UK Committee—Annual Report 1970-71. Pp. 12. (London: Freedom From Hunger Campaign, 1972.) [131]

Young Fabian Pamphlet No. 28: The Universities—Pressures and Prospects. By Colin Crouch and Stephen Mennell. Pp. 47. (London: Fabian Society, 1972.) 45p. [131]

Necks, Holes and Crazes: Inaugural Lecture delivered in the University of Birmingham, 25 May 1971. By Professor R. N. Haward. Pp. 19. (Birmingham: The University, 1971.) 25p. [141]

Particles and Resonances in Modern Physics. By Professor P. G. Burke. (An Augural Lecture delivered before the Queen's University of Belfast on 11 December 1968. (New Lecture Series No. 49.) Pp. 28. (Belfast: The Queen's University, 1969.) 40p. [141]

Department of the Environment—Building Research Station. European Plumbing Notes—a Series of Papers on Aspects of Water Services and Drainage in Europe. 8: Comparative Summary and Index. Pp. 32. (Garston, Watford: Building Research Station, 1971.) [141]

PEP. Broadsheet 532: Making Labour Markets Work—a Comparison of the UK and Swedish Systems. By Santosh Mukherjee. Pp. x+153. (London: PEP, 1972.) £2. [171]

Building Research Station Digest No. 137: Principles of Joint Design. Pp. 4. (London: HMSO, 1972.) 5p. [171]

The Royal Society. Evaluation of the Potential United Kingdom Programme for the Geodynamics Project, 1972-1977. (London: The Royal Society, 1971.) [171]

Council for Scientific Policy. Report of the Working Group on Scientific Interchange. Pp. v+55. (Cmd. 4843.) (London: HMSO, 1972.) 42p net. [171]

Hedges and Local History. Pp. 36. (London: National Council of Social Service, 1972. Published for the Standing Conference for Local History.) 50p. [181]

Science and Coal—The Future. By Lord Robens. (The Twentieth Coal Science Lecture delivered in London, Monday, 11th October, 1971.) Pp. 10. (London: Assistant Director of Administration, Research and Development Department, National Coal Board, Hobart House, Grosvenor Place, 1972. Published on behalf of BCURA Council.) [191]

Smallpox Inoculation: An Eighteenth Century Mathematical Controversy. Translation and Critical Commentary by L. Bradley. Pp. 72. (Nottingham: Adult Education Department, The University, 1971.) 75p. [191]

Department of Education and Science. Statistics of Education 1970. Vol. 2: School Leavers, C.S.E.

and G.C.E. Pp. xxiv+82. (London: HMSO, 1972.) £1.60 net. [191]

BBC Handbook 1972. (London: BBC, 1972.) 50p. [201]

The Polluters: Industry or Government? Essay 1 by Neil H. Jacoby. Essay 2 by F. G. Pennance. Pp. 55. (London: The Institute of Economic Affairs, 1972.) 40p. [201]

The Zoological Record. 1969, Vol. 106, Section 6c: Vermes. Part C: Conodonts, Fossil Vermes, Trace Fossils. Compiled by S. Ware. Pp. 25. £3.50; \$8.50. 1969, Vol. 106, Section 17: Reptilia. Compiled by the Staff of the Zoological Society of London. Pp. 112. £6.50. (London: The Zoological Society of London, 1971.) [201]

Computers in Water Resources Management. By A. R. Gourlay. Pp. 33. (Peterlee, County Durham: UK Scientific Centre, IBM United Kingdom Limited, 1971.) [211]

University of Cambridge, Department of Applied Biology. Memoir No. 43: A summary of the papers published and research in progress by the members of the Department and its Associated Research Organizations during the period October 1st, 1970—September 30th, 1971. Review Series No. 26: Animal Production—a Study of Animal Systems. Pp. 20. (Cambridge: University of Cambridge, 1971.) 15p. [211]

Energy in the Seventies. By Professor Frank S. McFadzean. Pp. 40. (Glasgow: University of Strathclyde, 1972.) [211]

Department of Education and Science. Teacher Education and Training. (Report by a Committee of Inquiry appointed by the Secretary of State for Education and Science, under the Chairmanship of Lord James of Rusholme.) Pp. viii+128. (London: HMSO, 1972.) 85p net. [211]

Inner London Education Authority. Children with Special Difficulties. (A report by the Education Officer to the Education Schools Sub-Committee, 8th July 1971.) Pp. 37. (London: Inner London Education Authority, 1972.) [211]

Fisons Grassland Research—a Report to the Farmer. Pp. 30. (Felixstowe, Suffolk: Fisons Limited, 1972.) [211]

## Other Countries

US Department of Commerce: National Bureau of Standards. NBS Technical Note 270-6: Selected Values of Chemical Thermodynamic Properties. Tables for the Alkaline Earth Elements (Elements 92 through 97 in the Standard Order of Arrangement). By V. B. Parker, D. D. Wagman and W. H. Evans. Pp. xiii+106. (Washington, DC: Government Printing Office, 1971.) \$1.25. [131]

Institut Royal Météorologique de Belgique. Publications, Série A, No. 73: Les Pointes Maximales du Vent en Belgique. Par R. Sneyers. Pp. 32. (Eccle-Bruxelles: Institut Royal Météorologique de Belgique, 1971.) [131]

Glossary of Terms and Symbols in Thermionic Conversion. (Joint ENEA-IAEA Liaison Group on Thermionic Electrical Power Generation.) Pp. 115. (Paris: OECD, European Nuclear Energy Agency, 1971.) 23 francs; £1.75; \$5. [131]

Australian Journal of Zoology. Supplementary Series, No. 8: The Australian Scatopsidae (Diptera). By E. F. Cook. Pp. 90. (East Melbourne: Editorial and Publications Section, CSIRO, 1971.) [131]

Smithsonian Contributions to Paleobiology, No. 6: Functional Morphology and Biofacies Distribution of Cheilostome Bryozoa in the Danian Stage (Paleocene) of Southern Scandinavia. By Alan H. Cheek. Pp. iv+87. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) \$1.50. [131]

United States National Museum Bulletin No. 298: Publications of the United States National Museum (1947-1970). Pp. 77. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) 60 cents. [131]

Carnegie Endowment for International Peace. International Conciliation—Issues Before the 26th General Assembly. Pp. 222. (New York: Carnegie Endowment for International Peace, 1971.) \$2. [141]

US Department of the Interior: Geological Survey. Bulletin 1319-F: Mineral Resources of the Glacier Primitive Area, Wyoming. (Studies Related to Wilderness—Primitive Areas.) By H. C. Granger, E. J. McKay, R. E. Mattick, Lowell Patten and Paul McIlroy. Pp. vii+113+2 plates. Professional Paper 497-E: Geology of the Compacting Deposits in the Los Banos—Kettleman City Subside Area, California. By R. E. Miller, J. H. Green and G. H. Davis. Pp. iv+46+5 plates. Professional Paper 668: Hybrid Granitoid Rocks of the Southern Snake Range, Nevada. By Donald E. Lee and Richard E. Van Loenen. Pp. iv+48+1 plate. Professional Paper 706: Pennsylvanian Fusulinids from South-eastern Alaska. By Raymond C. Douglass. Pp. iii+20+7 plates. 70 cents. Professional Paper 750-B: Geological Survey Research 1971, Chapter B. Pp. v+282. \$3.50. (Washington, DC: Government Printing Office, 1971.) [141]

Bulletin of the American Museum of Natural History. Vol. 145, Article 4: A Revision of the Nacophorini from Cool and Cold Temperate Southern South America (Lepidoptera, Geometridae). By Frederick H. Rindge. Pp. 303-392. \$3.70. Vol. 146, Article 1: Caspian-Like Relict Molluscan Fauna in the South American Permian. By Bruce Runnegar and Norman D. Newell. Pp. 1-66. \$3.25. (New York: American Museum of Natural History, 1971.) [171]

Policies and People—First Annual Conference and Annual General Meeting of SCITEC, Ottawa, Ontario, June 28, 29, 1971. Pp. 79. (Ottawa: SCITEC, 1971.) [171]

Smithsonian Contributions to Zoology, No. 111: Systematic Notes on a Collection of Birds from Kenya. By S. Dillon Ripley and G. M. Bond. Pp. 21. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) 35 cents. [171]

Deutsche Forschungsgemeinschaft (German Research Association): Farbstoff-Kommission (Dye-Stuff Commission). Mitteilungen 3 (3rd Report)—Colours for Cosmetics. Third edition published 1968. English translation of the "Introduction and Registers of Colours" published 1971. Pp. 32. (Bonn—Bad Godesberg: Deutsche Forschungsgemeinschaft, 1971.) [171]

US Department of the Interior: Geological Survey. Water-Supply Paper 1535-P: Chemical Comparison of Atmospheric Precipitation in the North-eastern United States. By F. J. Pearson, Jr., and Donald W. Fisher. Pp. iv+23. 25 cents. Water-Supply Paper 1934: Surface Water Supply of the United States 1961-65. Part 13: Snake River Basin. Pp. x+776. \$3.75. (Washington, DC: Government Printing Office, 1971.) [171]

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Forest Products—Research Review 1970-71. Pp. 64. (Melbourne: CSIRO, 1971.) [171]

SHF and EHF Propagation in Snowy Districts. Edited by A. Matsumoto and A. Nishitsuji. (Monograph Series of the Research Institute of Applied Electricity, No. 19, 1971.) Pp. 103. (Sapporo, Japan: Research Institute of Applied Electricity, Hokkaido University, 1971.) [181]

Global Environmental Monitoring—a Report submitted to the United Nations Conference on the Human Environment, Stockholm 1972, by the Commission on Monitoring of the Scientific Committee on Problems of the Environment (SCIEP) of the International Council of Scientific Unions (ICSU). Pp. 67. (Stockholm: Swedish Natural Science Research Council, Wenner-Gren Centre, Box 23136, 1971.) \$2. [191]

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